

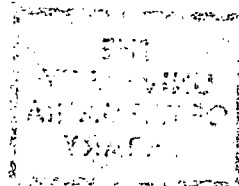
STUDIES IN THE PHYSIOLOGICAL - GENETICS OF FLOWERING IN PISUM.

by

Ian Campbell Murfet

submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy



UNIVERSITY OF TASMANIA


HOBART

January, 1970.



DECLARATION.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and contains no copy of paraphrase of material previously published or written by another person, except where due reference is made in the text.



(IAN C. MURFET)

ACKNOWLEDGEMENTS

My thanks go to Professor H.N. Barber, F.R.S. under whose supervision this work was commenced and whose contribution to the physiological-genetical approach I wish to acknowledge; to Professor W.D. Jackson and Mr. C. Pearson who designed and built the large-scale controlled-photoperiod facilities which made this type of genetic analysis possible; to those who provided technical assistance at various times, in particular Mr. R. Buckney, who helped with a large section of the programme; to Dr. M. Whitten and members of the Genetics Section, C.S.I.R.O. Division of Plant Industry, Canberra for helpful discussion; to Professor W.D. Jackson and Dr. R.K. Crowden for critical reading of the manuscript; to my wife for assisting with a section of the practical work and typing the manuscript; to the Rural Credits Development Fund and the University of Tasmania for grants in aid and to the C.S.I.R.O. for a Studentship which enabled me to commence this work.

TABLE OF CONTENTSPage
Number.

Acknowledgements.	III
List of Figures and Tables.	IX
Abbreviations.	X

PART I.Introduction.

<u>CHAPTER 1. Review.</u>	2
---------------------------	---

PART II.The crosses and genetic analysis.CHAPTER 2. Materials, methods and a phenotypic classification.

Growing conditions.	6
The characters recorded.	7
The varieties.	8
Varietal classification into phenotypic classes ED, EI and L.	9
Figure 1.	12

CHAPTER 3. The results in brief presented point by point.

The major genes.

The theory - dominant genes S_1 , E and S_2 and their interaction.	13
The crossing programme.	13
Individual segregation of gene pairs S_1/s_1 , E/e and S_2/s_2 .	13
Joint segregation of S_1 , E and S_2 with various markers.	14
Development of the theory.	14
Maternal influence - particularly on the expression of gene S_1 .	15
Pleiotropy - the multiple effects of gene S_2 .	15
Penetrance of gene S_2 as influenced by polygenes and environment.	17

CHAPTER 3. The results in brief presented point by point (Continued)

Within-class variation.

New combinations and dosages of the major flowering genes.	17
Pleiotropy of major genes not primarily concerned with flowering.	18
Various quantitative systems.	18
Vegetative reversion in EI plants and the influence of length genes.	19
Tables 1, 2, 3 and 3a.	21

CHAPTER 4. The results in detail analysed one cross at a time.

Cross 26 (L2 x L8) Segregation of S_1/s_1 on an ES_2 background.	25
Between-class variation.	25
Linkage of A and S_1 .	26
Within-class variation.	26
Cross 20 (L59 x L53) Segregation of E/e and S_2/s_2 on an s_1 background.	26
A clear-cut segregation in F_2 into 4 ED : 9 EI : 3 L and genotypic analysis of this F_2 by progeny testing.	27
Within-class variation as influenced by new combinations and dosages of major genes.	28
Some further remarks on the phenotypic differences between classes.	29
The economics of labour, time and space.	30
Cross 57 (L2 x L53) Segregation of S_1/s_1 and E/e on an S_2 background.	30
Segregation of the major genes.	31
Polygenic modification of the penetrance of S_2 .	33
Crosses 119 and 121 (L61 x L22) Segregation of S_2/s_2 on an s_1e background.	34
Free recombination between A and S_2 shows that S_1 and S_2 are not allelic.	34
Factors influencing the penetrance of genotypic $s_1s_1eeS_2-$.	35
Within-class variation and quantitative genes.	37

<u>CHAPTER 4. The results in detail analysed one cross at a time</u>	(Continued)
Cross 50 (L22 x L53) Segregation of S_2/s_2 on an s_1e background.	38
Development of L58, a pure line of genotype s_1es_2 .	38
Cross 40 (L24 x L53) Segregation of S_1/s_1 on an eS_2 background.	39
A further example of incomplete penetrance in genotype $s_1s_1eeS_2S_2$.	39
Cross 2 (L8 x L24) Segregation of S_1/s_1 and E/e on an S_2 background.	39
Between-class variation.	39
Within-class variation.	
Dosage of E .	40
Pleiotropy of Cy_1 .	40
Cross 125 (L58 x L24) Segregation of S_1/s_1 and S_2/s_2 on an e background.	41
The first appearance of genotype $S_1-ees_2s_2$.	41
Maternal influence on the expression of gene S_1 .	41
Future work.	43
Cross 53 (L22 x L2) Segregation of S_1/s_1 and S_2/s_2 on an E background.	44
The first appearance of genotype $S_1-EEs_2s_2$.	44
Cross 114 (L60 x L53) Segregation of E/e on an s_1S_2 background.	46
Linkage of genes E and \underline{P} .	46
Cross 126 (L8 x L53) Segregation of E/e on an s_1S_2 background.	47
Further evidence of linkage between E and \underline{P} .	47
Cross 127 (L58 x L8) Segregation of E/e and S_2/s_2 on an s_1 background.	48
Late segregates derived from two early parents.	48
Figure 2 and Tables 4, 5+6, 7, 8, 9, 9a, 10, 11 and 12.	50

CHAPTER 5. General discussion of the genetics.

On the relationship of symbols $\underline{L_f}$, $\underline{S_n}$, S_1 and S_2 .	60
Theoretical schemes for flowering control.	63
A speculative model of gene action.	
Action of $\underline{Cy_1}$.	65
Action of S_2 .	66
Action of E.	67
Action of penetrance modifiers of gene S_2 .	69
Action of S_1 .	69
Conclusion.	71

PART III.A physiological experiment testing the proposed model of gene action.CHAPTER 6. Intervarietal grafting with known genotypes.

Introduction.	74
Materials and methods.	74
Results.	75
Self-grafts.	77
Grafts using scions of genotype s_1s_2 .	77
S_1S_2 scions 2 and 24 on stocks 59 and 58.	79
s_1S_2 scions 60 and 53 on stocks 59 and 58.	80
S_1S_2 scions 2 and 24 on stocks 60 and 2.	81
s_1S_2 scions 60 and 53 on stocks 60 and 2.	81
Scions S_1S_2 and s_1S_2 on stocks 24 and 53.	81
Slow grafts.	82
Conclusion.	83
Figures 3 and 4 and Table 13.	86

PART IV.General discussion.CHAPTER 7. The proposed model in relation to other published work.

Papers suggesting a flower inhibitor in peas.	93
Papers favouring a promotor-only system in peas.	98
Some examples from other plants.	103
Concluding discussion.	104
The Balance Model.	107
Summary.	108
References.	112

LIST OF FIGURES and TABLES.Page Number.

Figure 1	11, 12
" 2	49, 50
" 3	85, 86
" 4	87, 88
Table 1	21
" 2	22
" 3	23
" 3a	24
" 4	51
" 5	52
" 6	52
" 7	53
" 8	54
" 9	55
" 9a	56
" 10	57
" 11	58
" 12	59
" 13	89 - 91

ABBREVIATIONS.

- LD - long day photoperiod.
- SD - short day photoperiod.
- flowering time - time in days from sowing to first open flower.
- flowering node - the first node at which a flower bud is initiated. The cotyledonary node is taken as zero.
- ϕ - florigen.
- K - colysanthin (flower inhibiting substance).
- L58 - Line 58 .
- cf - compare with .
- cv - cultivar .
- KR - cv Kleine Rheinlanderin .
- AL - cv Alderman .
- M - cv Massey (Line 22) - probably contains
genotypes $s_1s_1E-s_2s_2$ and $s_1s_1ees_2s_2$. Line 59
(s_1Es_2) is a pure selection from this cultivar. } See p. 8.
and footnote
on p. 93.
- G - cv Greenfeast - consistency in doubt but
probably genotype S_1eS_2 . Line 24 is a pure
selection from this cultivar.
- ED - early developing class - standard variety L58
(genotype s_1es_2). See p. 8,9.
- EI - early initiating class - standard variety L60
(genotype s_1ES_2). See p. 8,9.
- L - late class - standard variety L24 (genotype S_1eS_2). See p. 8,9.
- \bar{x} - mean .
- h^2 - heritability coefficient .
- RCV - recombination value.

- P - probability.
- x - $P < 0.05$.
- xx - $P < 0.01$.
- xxx - $P < 0.001$.
- χ^2_1 - Chi-squared with 1 degree of freedom.
- t_{18} - Students t with 18 degrees of freedom.

PART I.

INTRODUCTION.

CHAPTER I.

Review.

Mendel (1865) found the flowering time of pea hybrids to stand almost exactly between the times of the two parents. Since that time, numerous papers have been written on the subject including one by Rasmusson (1935) in which he called for "co-operation between genetical and physiological research". Barber (1959) used such a joint approach to the subject and the present work is part of a programme which follows his techniques, whereby physiological information is used to help detect genetic segregation and known genotypes may in turn be used in experiments to investigate the physiological action of the various genes.

Recent findings have suggested that flowering is basically controlled by a single major gene and one or more systems of polygenes, and that for the most part flowering time and flowering node are determined by the same genes. Barber (1959) has proposed that late varieties differ from early varieties by possessing a dominant gene Sn which "has three pleiotropic effects on flowering - a delaying action and the induction of competence to respond to vernalisation and photoperiod". He suggested that the Sn gene causes these effects by producing a flower delaying substance (colysanthin) which must be destroyed before flowering can take place. In addition to the major control by the Sn locus, Barber proposed two other gene systems controlling flowering. The first is a system of genes modifying the action of the Sn gene. The second is a system of polygenes which alter the node of first flower by a physiological mechanism other than by way of colysanthin. Few workers have attempted an extensive array of inter-related crosses but Rowlands (1964) has investigated flowering in diallel crosses involving 7 varieties. He proposed that a simple polygenic system is primarily responsible for the control of flowering with a major gene (Sn) or "effective factor" which is dominant for a delay in flowering

and whose effect is increased during short days. Barber recorded node of first flower and Rowlands flowering time, but the similarity between the results is even closer than Rowlands realised, for like Knavel (1967) he was under the impression that Barber had suggested the Sn gene operated 'only by the induction of vernalisation and photoperiodic responses'. It is clear from the quotation above that Barber also ascribed to Sn a general delaying effect.

Von Tschermak (1910), Hoshino (1915) and Wellensiek (1925a) have explanations in terms of two major genes but for reasons previously discussed by Clay (1935) these proposals are unconvincing. Most workers have only measured either flowering node or flowering time, but those who have measured both variables, report a strong correlation between the two, e.g., Tedin (1897), Wellensiek (1925a) and Rowlands (1964). Paton and Barber (1954) confirmed this correlation but found some varieties to lie well away from the regression line. Hansel (1954) paid particular attention to the relationship between node and time. He found that although the flowering time of the F_2 plants and F_3 families was undoubtedly determined above all by the node-number, certain F_3 families occurred in which the flowering time was too long or too short for their node number. In order to explain both the general high correlation of node-number and flowering-time and the exceptions Hansel assumes two "Gengruppen" whose main factors are recombinable. "Gengruppe" B determines the position of the flower primordium and the rapidity of floral development and "Gen(gruppe)" D which modifies the speed of floral differentiation.

F_2 distributions for flowering node and time have usually been continuous. However, both Oppenheim (1921) (after Bot. Abst.) and Barber (1959) have obtained discontinuous bimodal distributions for flowering node. Tedin and Tedin (1923) also obtained one distribution which was almost discontinuous. In each case the numbers of early and late plants were consistent with a single factor difference with dominance of late. F_3 data are not given. The Tedins named

the gene for high node number Sn and this symbol was used by Barber.

All those who have made crosses which segregated for both flowering time and the basic gene for flower colour (gene A) report a relationship between the two no matter what form the F_2 distribution has taken (Lock (1907), von Tschermak (1910), Hoshino (1915), Rasmusson (1935) and Hansel (1954). Rasmusson (1935) made crosses between early-white and late-red and vice versa. He proposed that part of the F_2 variation in flowering time could be explained on the segregation of a near dominant late gene Xa (he estimated one Xa having an effect equal to 80% of two Xa's) which was linked to the A-gene for flower colour. He found that Xa and the le gene for internode length (or closely linked factor) were responsible for about half the genic variation in F_2 flowering time and that the other half was probably due to modifiers. The le gene was partially dominant for a delay in flowering time. Other workers have investigated the interaction between length factors and flowering but contradictory findings are reported for the pleiotropic effect of Le on flowering. Barber (1959) presents strong evidence of a consistent cross pleiotropic effect of the flowering gene Sn on internode length.

The physiology of flowering in peas has been extensively investigated and is the subject of a recent review by Haupt (1969) which removes the need for a detailed review here. There are two main schools of thought on the subject which arise not so much from a difference in results but a difference in interpretation of the results. Barber and his associates (Paton and Barber (1955), Sprent and Barber (1957), Barber (1959), favour an explanation in terms of a flower inhibitor. Lates are late because the Sn gene produces colysanthin which is preferentially destroyed by long days and low temperatures and which is absent from earlies. Haupt supported by Kohler (1965) argues in favour of a promotor-only scheme. Earlies are early because they possess florigen which is suppressed in lates by the Sn gene. This subject is discussed at length in Chapter VII.

PART II.

THE CROSSES AND GENETIC ANALYSIS.

CHAPTER 2.

Materials, methods and a phenotypic classification.

Growing conditions. The peas were grown in 6 lb. tin cans and plastic boxes in a 50/50 by volume mixture of quarter inch dolerite chips and vermiculite. Nutrient solution in the form of a modified Hoagland's solution was supplied once a week. Our controlled environment facilities provided good control over the length of the light period but only limited control of temperature. The plants were grown on trucks 14 feet long with supports for plants 8 feet tall. SD trucks moved automatically in and out of the dark compartments at prescribed times. A system of heaters and fans maintained the same temperature in the LD and SD compartments. The heaters ensured that the temperature remained high enough to eliminate the possibility of vernalisation. No cooling was provided apart from glasshouse vents which opened automatically at a pre-determined temperature to allow a cross-flow of outside air. LD's were supplied by supplementing natural photoperiod with banks of incandescent and fluorescent lights. These lights could be raised or lowered and were usually adjusted to supply a meter reading of 40 ft. candles at plant height. With this system of photoperiod control, the period of natural light received by the LD treatments varies throughout the year. Therefore, genuine photoperiod effects may be confounded with photo-dependent effects which arise from an increased level of photosynthesis.

Seeds were not sterilised and except^{for} the first few plantings fungicidal seed dressing was not employed. Fresh vermiculite/dolerite growth-medium was used for each batch of plants. The percentage of seeds planted which survived through to harvest and scoring was normally around 98 - 100 %. The testa on seeds from mothers carrying the A gene was sometimes very impervious, e.g., in the F₂ of Cross 57 some seeds were found to be bullet-hard after lying for three

weeks in wet growing medium. Delayed and irregular germination is particularly to be avoided where flowering time is under consideration. Accordingly, the testa of all seeds in crosses involving the A allele were nicked with a razor blade before sowing. Prompt and regular germination followed as a result.

The characters recorded. Flowering behaviour was measured in terms of two variables - flowering time (days from sowing to first open flower) and flowering node (the first node at which a flower bud is initiated, the cotyledonary node taken as zero). Wherever these two general terms are used in this report the precise meaning in brackets is inferred. Data were recorded from main shoots only. Under the glasshouse conditions employed, laterals very rarely grew in LD's but a lateral sometimes developed from one or two of the lower nodes under SD's particularly with cooler temperatures. Plants were inspected regularly and any laterals cut off. To record flowering node, the plant was first checked to make sure it was a main shoot and that all basal nodes were present including the two with scale leaves. Every node from the base up was then inspected to detect the first node at which a flower had been initiated, i.e., the first node to carry a flower primordium irrespective of the degree of post-primordial development. Once the pea apex switches to producing flower primordia, it normally continues in the flowering state, but it can revert to producing vegetative buds, particularly after certain treatments (Barber (1959) and Kohler (1965)) and the sequence flowering-vegetative-flowering can result. In the present experiments all nodes above the first flower were checked for vegetative reversion.

Initiation of a flower primordium is not always followed by the growth of the primordium into a bud and the bud into a fully developed flower. The genetic analysis which follows depends in part upon this point. The flower primordium almost invariably developed into a small bud but short days fully suppressed the development of the first few flower buds in certain genotypes which could therefore be distinguished from those genotypes in which the first

flower buds always developed into mature flowers. Smooth axils without any bud were extremely rare in these crosses and possibly resulted from very early abortion of a flower primordium. If the flower bud failed to develop further it usually remained visible in the axil until harvest. If the bud itself did fall off, the thin stalk was clearly distinguishable from the squat vegetative buds which remained dormant at the non-flowering nodes.

The varieties. The peas used in this work come from what some taxonomists have considered as two species - P. sativum and P. arvense. The taxonomy of the genus is discussed by Wellensiek (1925) and Lamprecht (1956). All crosses were of normal fertility. Line 2. (Graue niedrige) is a dwarf, late, grey-pea obtained from Dr. R. Lamm at Alnarp. Other names are Lamm Line 2 and Rasmusson Gd. Line 8 is an early, cryptodwarf type obtained from Lamm (his Line 8a). Line 22 (Massey) is a dwarf, early type developed from a sample of twelve seeds of the local commercial garden-pea Massey. (This variety has proved to be heterogeneous for the E, e pair of genes uncovered in this work). Line 24 (Greenfeast) a dwarf late garden-pea developed from local commercial stocks by four generations of single-plant selection. Line 53 is a dwarf late obtained by several generations of single-plant selection from a late segregate in the F₂ of a cross between Lines 7 and 22. Line 59 is a single-plant selection direct from Line 22. (It is homozygous for EE). Lines 58, 60 and 61 were selected from crosses involving the parental lines described above. They are still under development at the time of writing and are maintained in individual pedigrees, not in bulk. They are not yet fixed for all genes, but they are pure for the major flowering genes and for convenience have been prematurely assigned line numbers. Line 58 is an early dwarf selected from Cross 50 (Line 22 x Line 53). Line 60 is a red-flowered, early dwarf selected from Cross 57 (Line 2 x Line 53). Line 61 is a red-flowered, late dwarf selected from Cross 57.

Varietal classification into phenotypic classes. Flowering time and flowering node data for the above varieties are given in Fig. 1 (p. 12) along with data for the more extreme varieties in our stocks. Lines 22, 60 and 61 are not shown but their values are close to those of Lines 59, 8 and 53 respectively. Twelve plants of each variety were grown under long days of 18 hours and short days of 8 hours. The variance for pure varieties is usually small and was in fact zero for the flowering node of Lines 8, 58 and 60 in short days. As a result, although some points are fairly close together, in a statistical sense, every point on the figure differs significantly from every other point in terms of at least one co-ordinate. Biologically, there are some very marked similarities as well as differences in flowering behaviour which enable the varieties used in the present investigation to be placed into three distinct phenotypic classes. The classes may be defined in terms of response to photoperiod. However, a definition in terms of the flowering behaviour in short days and the relationship of the SD co-ordinates to those of a standard variety is more convenient. The smaller but significant within-class differences receive some consideration during the genetic investigation, but attention is concentrated on the between-class differences.

Class ED (early developing). There are two diagnostic features of this class:- flowering node and time are unaffected by photoperiod and both characters are early under SD's. Examples are Lines 22, 58 and 59. Line 59 is taken as the standard variety. The first initiated flower primordium develops through to a mature flower in both photoperiods. Actually ED varieties flowered at a slightly earlier time under SD's (e.g., Line 58 was 1.08 ± 0.34 days earlier, $t_{22} = 3.21^{xx}$) but there is some doubt as to whether this effect is due to photoperiod or a slight temperature difference between the compartments. The difference itself is minute compared with the time shift shown by members of the EI and L classes (cf Line 8 17.25 ± 1.22 days, $t_{22} = 14.13^{xxx}$).

Class EI (early initiating). Flowering time is delayed by SD's but flowering node is unaffected by photoperiod. Flowering node is early under LD's or SD's and flowering time is early under LD's but late under SD's. Examples are L8 and L60. Line 60 is taken as the standard variety. The late flowering time in SD's results from the failure of the first few flower buds to develop into mature flowers. For example, in the present experiment the first 8.92 ± 0.78 flower buds aborted under SD's in the case of L8. EI and ED plants give similar flowering data under LD's.

Class L. (late) In this class, both flowering time and node are delayed by SD's under which photoperiod both characters are late. Examples are Lines 2, 24, 53 and 61. Line 24 is taken as the standard variety. Under LD's plants of class L are later than plants of classes ED and EI.

The extreme varieties. Lines 7, 16 and 63 were not used in the present crosses. They were included here for comparison because they illustrate genetic types which cannot be obtained by recombination from the gene pool used in the present crosses. Line 7 reacts like an EI type but flowers at nodes 6 and 7. No plant in the present crosses ever flowered earlier than node 8. There is clearly a substantial difference between Line 7 and the EI plants in the present crosses. Lamprecht (1956b) describes a still earlier type which flowers out of nodes 2 and 3. Line 63 would pass for an L type under LD's but the response to SD's is much greater than the response shown by the L-type parents or segregates in the crosses. For example, the time shift for Line 24 is 14.58 ± 0.54 days but for Line 63 it is 48.55 ± 0.67 days. Line 16 shows a similar very large response to short days, but is also substantially later than L plants under LD's. Lines 63 and 16 may have as many as 70 vegetative nodes and the latter may grow to a height of 6 metres. Such plants are not easily accommodated in our controlled environment equipment.

Fig. 1. The mean node of first flower and flowering time for several varieties under short days (8 hours) and long days (18 hours). The arrow points towards the SD coordinates. The average standard errors are too small to show graphically. They are for flowering node LD ± 0.15 nodes and SD ± 0.32 nodes and flowering time LD ± 0.27 days and SD ± 0.59 days. Data are not given for Lines 22, 60 and 61 as they correspond very closely to the data for Lines 59, 8 and 53 respectively. Line 16 was not grown at the same time as the other eleven varieties.

CHAPTER 3.

The results in brief presented point by point.

The aim of this chapter is (a) to introduce the reader to the genetic model and the sequence of events leading to this model and (b) to provide a point by point outline of the main results. For convenience of presentation, many details are deferred to Chapter 4, where the results are analysed in detail, one cross at a time.

The major genes.

The Theory. Three dominant major genes S_1 , E and S_2 are proposed to interact as follows. The triple recessive is an ED type. Addition of S_2 creates an L type. E is epistatic to S_2 in terms of flowering node and genotype s_1ES_2 is an EI type. S_1 is epistatic to E and S_1ES_2 is again L type as is S_1eS_2 . S_1 and E have little or no effect by themselves and genotypes S_1es_2 , s_1Es_2 and S_1Es_2 are essentially ED.

The crossing programme. The parents, their proposed genotypes, the crosses made and the expected ratios are shown in Table 1 (p.21). It is clearly necessary to go beyond F_2 to validate these ratios and check on misclassification. The different crosses are not all investigated to the same depth. In Cross 20 an entire F_2 has been genotyped by growing F_3 and one section was checked through to F_4 . In most other crosses (26, 57, 2, 114, 40, 125, 50 and 119) certain sections have been taken beyond F_2 and up to F_5 if necessary. In the remaining crosses (53, 126 and 127) time and space have not permitted the growing of generations higher than F_2 . However, I believe that sufficient material has been grown and the general agreement with expectation is good enough to permit a high degree of confidence in the theory.

Individual segregation of gene pairs S_1/s_1 , E/e and S_2/s_2 . Individual segregation data are given in Table 2 (p.22). Segregation of the S_1/s_1 pair

is typically Mendelian. Segregation of the E/e pair sometimes shows a deficiency of recessives (e.g. Cross 126) which is thought to result from impenetrance of genotype s_1eS_2 (classifies EI instead of L) a matter pursued further under the heading of minor genes. Where misclassification is rare (e.g. Cross 20) or revealed by progeny testing (e.g. Cross 114) segregation of the E/e pair is normal. Analysis of the S_2/s_2 segregations shows a small heterogeneity χ^2 and a very large deviation χ^2 indicating a significant disturbance. The shortage of recessives is not caused by differential survival as survival was usually better than 99 %. Nor is there any suspicion of impenetrance as the genotypes could be confidently distinguished by their flowering time and progeny testing gave no evidence of misclassification.

Joint segregation of S_1 , E and S_2 with various markers. The three pairs of alleles S_1/s_1 , E/e and S_2/s_2 segregate independently. The joint segregation data for the three major genes and various markers are given in Table 3 (p. 23). S_1 is linked to the A gene for anthocyanin with a recombination value of about 9 % and E linked to the P gene for pod membrane with a recombination value of about 22 %. The linkage of S_2 is not known as it recombines freely with the six markers tested (A, I, Cy₁, V, P and R).

Development of the theory. The three gene scheme may be established as follows. Cross 26 reveals a dominant late gene S_1 closely linked to the A gene for anthocyanin. Cross 20 establishes a dominant early gene E and a dominant late gene S_2 . Cross 57 confirms E and shows that S_1 and S_2 are not identical although they could be allelic. In Crosses 119/121, S_2 and A are segregating in coupling and free recombination between the two loci shows that S_1 and S_2 are separate loci. The difference in effect between S_1 and S_2 is revealed in Cross 125 where S_1 is seen to have little to no effect on its own depending on the genotype of the mother (See maternal influence section below). Only one genotype, S_1Es_2 , remains unseen at this point and Cross 53

is thought to confirm the anticipated ED nature of this type. The remaining crosses (2, 40, 50, 114, 126 and 127) are confirmatory. Crosses 114, 126 and 127 reveal the linkage of E and P. Cross 127 was the last cross to be made and at once provides a striking example of gene interaction and a confirmation of the theory. Both parents are early (ED x EI) and flower in the region of nodes 9 - 12, but as expected 3/16ths of the F_2 (4ED : 9EI : 3L) flower at a high node; some plants flowering as late as node 33.

Maternal influence. Influence of the mother plant on flowering node of the offspring is evident in several crosses. For example in Cross 126 two F_1^s (genotype $s_1s_1EeS_2S_2$) were generated by direct reciprocal crossing between single plants but there is a significant difference in the flowering node of the reciprocal samples (1.20 ± 0.31 nodes $t_{18} = 3.88^{xx}$). No difference was found between the F_2^s descended from these F_1^s . The same remarks apply to the F_1 of Cross 125 although a different genotype ($S_1s_1eeS_2s_2$) is involved. The most striking effect of maternal influence is seen in the F_2 and F_3 of this cross. The ED class in F_2 contains genotypes $S_1S_1ees_2s_2$, $S_1s_1ees_2s_2$ and $s_1s_1ees_2s_2$ and all flower within the usual ED range. The same genotypes occur in F_3 but plants carrying S_1 now flower 2 - 4 nodes later. Environmental differences were shown not to be responsible by the use of a control F_2 . Evidently plants carrying S_1 are within the usual ED range when derived from mothers carrying S_2 but slightly later when derived from mothers lacking S_2 .

Pleiotropy - the multiple effects of gene S_2 . S_1 and E seem principally involved with the regulation of flowering node and have little or no effect in the absence of S_2 . In contrast S_2 has manifold effects. The effects on flowering behaviour may be seen Fig. 1 (p.12). Lines with dominant S_2 are:- L type - Lines 2, 24 and 53 and EI type - Line 8. Lines without S_2 are the ED types - Lines 58 and 59. In L plants S_2 invariably causes a delay in

flowering time. This effect is caused for the most part by a delay of flower initiation to a high node. In EI plants flowering node is not affected but flowering time is delayed under SD's by suppressed or retarded development of the lower flower buds. Actually S_2 does cause a slight but significant increase in flowering node in EI plants (see Cross 20) but this effect is an order of magnitude less than the delay in flowering time. S_2 confers on all plants the ability to respond to photoperiod in terms of flowering time but only L plants respond in terms of flowering node. It is not known for sure whether this ability is confined to plants with dominant S_2 as the reactions of genotypes S_1es_2 and S_1Es_2 have not been fully tested.

Plants with S_2 were always recognisably taller than plants lacking S_2 given the same background of length factors etc. In Cross 119 F_2 , L plants ($s_1s_1ees_2-$) were some 540 % taller than ED plants ($s_1s_1ees_2s_2$). The greater height comes very largely from an increase in the total number of nodes which in turn derives from a two-fold source, an increase in the number of vegetative or reproductively ineffective nodes and a prolongation of growth after effective flowering has commenced. The same two-fold source presumably accounts for the substantially higher yield in plants with gene S_2 . In Cross 20 F_2 the lowest yield for an L plant was 24 seeds and the highest yield for any ED plant was 11 seeds. Average yield was increased by over 600 %. (ED 5.9 ± 0.4 seeds, L 35.7 ± 1.9 seeds $t_{50} = 14.9^{xxx}$). Plants lacking dominant S_2 show a marked tendency to complete their life cycle quickly. The first flower bud initiated tends to grow and set even under adverse circumstances such as SD's and once the first pod begins to develop, new growth soon ceases. ED plants hardly ever had more than 3 pods under the growing conditions used in these experiments. Yield can be increased by changing the growth-medium, nutrient availability, spacing etc.

In general gene S_2 opposes flowering and senescence.

Penetrance of gene S_2 as influenced by polygenes and environment. As mentioned above, genotype s_1eS_2 may occur in both class L and class EI. The two classes are distinct so the matter is treated as a penetrance phenomenon and as the usual phenotype is L, EI plants are considered the impenetrant form. The gene S_2 is only impenetrant in the sense of flowering node as its presence is always revealed by late flowering time under SD's. Penetrance varies from cross to cross. In general, it is high in the crosses involving garden type peas (e.g. Cross 20 where it is about 0.97) and lower in crosses involving or descended from a field pea Line 2 (e.g. it falls below 0.5 in some families from Cross 57). Line 2 is suspected of supplying polygenic modifiers lowering penetrance. Crosses 119 and 121 show that dosage of major gene S_2 has no influence on the penetrance which is decided by the background level of penetrance modifiers and environmental influences. Selection for lines with high and low penetrance achieved a significant divergence over a single generation but variation of environment caused changes in penetrance level of an equal or even greater magnitude.

Within-class variation.

A sample from a genetically pure line of peas shows only a small variation in flowering behaviour; for example, 2 - 3 nodes for early lines and 3 - 6 nodes for late lines. This variation is presumably governed by environmental differences and the homeostatic capacity of the genotype. An F_2 usually shows much greater variation within classes than the parents or genetically homogeneous F_1 . This is particularly true of the late class as illustrated by Cross 26 (Table 4, p. 51). Several sources of increased within-class variation are illustrated by these crosses.

New combinations and dosages of the major flowering genes. Partially and completely genotyped F_2^s of Cross 20 are given in Tables 5 and 6 (p. 52). Considering flowering node (See Table 5) the range for the early class expanded

from 2 nodes for the parent to 8 nodes for the F_2 . Much of this variation comes from the appearance of a new combination of major genes, namely $E-S_2-$. (All plants in Cross 20 are s_1s_1). Of course, this genotype creates a class of EI plants different from either parent but when only flowering node is considered EI and ED plants constitute a single class. Again, where flowering time is considered EI and L plants form a single late class, but gene combination $E-S_2-$ flowers on the average, a little earlier than eeS_2- . Within the $E-S_2-$ sub-group (see Table 6) variation in dosage of E has a significant effect on flowering node. (This effect is seen again in Cross 2). In contrast, gene S_2 shows no dosage effect on flowering node in the EI class, but a dosage effect for S_2 is observed within the L class. (The last two observations are confirmed in Crosses 119/121).

Whether the significant difference in flowering behaviour between the three late lines 2, 24 and 53 is due to their differences in major gene content or to polygenes is not known. However, the two lines with dominants S_1 and S_2 are later than Line 53 which carries only dominant S_2 and this suggests that major gene S_1 may be at least partly responsible for the additional lateness.

Pleiotropy of major genes not primarily concerned with flowering. The major internode-length gene Cy_1 is segregating in Cross 2 giving a ratio of 3 dwarf : 1 cryptodwarf. Flowering node is also segregating giving the ratio 13 L : 3 EI. In both the L and EI classes, segregation of the Cy_1/cy_1 pair has a significant effect on flowering node (see Table 3a, P.24). Dominant Cy_1 tends to raise the flowering node of EI plants but the same gene tends to lower the flowering node of L plants.

Various quantitative systems. There are clearly cases in the present data where a quantitative component is indicated, as neither environmental differences nor the factors described above seem adequately to account for the variation observed. For example, in the late class of Crosses 26 (Table 4, p 51)

and 20 (Tables 5 and 6, p. 52) it is suspected that quantitative genes are segregating which operate either by specifically modifying the action of the major flowering genes or via some mechanism independent of the major genes or possibly via both methods. Data from late classes suitable for testing these ideas are not available as space was devoted primarily to establishing the major genes.

Considerable transgression of parental limits is common in late classes particularly at the early end (e.g., Cross 26). ED plants also show transgression but to a much smaller extent particularly on the early side, where the class is virtually bounded at node 9. The ED plants of Cross 119/121 illustrate these points (see Table 9, p. 55). Although they show only a small increase over the parental range (parent 2 nodes, F_2 3 nodes and F_3 5 nodes) a regression analysis of F_3 progeny mean on F_2 flowering node gives a highly significant heritability coefficient ($h^2 = 0.38 \pm 0.12$). These ED plants lack all three major dominants. The EI plants in Cross 119/121 are impenetrant L-type plants (genotype $s_1s_1eeS_2^-$) and a regression analysis in this case indicated negligible genetic control of flowering node within the group.

Vegetative reversion in EI plants and the influence of length genes.

ED and L plants almost invariably continue in the flowering state once the first flower bud is initiated. In contrast EI plants, whether genuine EI plants of genotype $s_1s_1E-S_2^-$ or impenetrant L plants of genotype $s_1s_1eeS_2^-$, show a marked tendency towards vegetative reversion. For example, in Cross 126 F_2 27 % of EI plants showed vegetative reversion for at least one node. Patterns ranged from a typical case such as 11F - 14F, 15V, 16F - ∞ , to an extreme case such as 13F, 14F, 15V - 27V, 28F - ∞ . The number of plants which show vegetative reversion was influenced in Cross 126 F_2 by the background of internode-length genes. The F_1 was of genotype $1eleCy_1cy_1cy_2^Ccy_2^S$. In F_2 21 % of dwarf ($1eleCy_1^-$ --) segregates showed vegetative reversion for one or

more nodes. With cryptodwarfs ($lelecy_1cy_1cy_2^c-$) 44 % showed reversion. The difference is significant at the 0.1 % level ($X_1^2 = 18.95^{xxx}$). Reversion was most frequent amongst the slender plants ($lelecy_1cy_1cy_2^scy_2^s$) but the rate of 56 % is not significantly higher than that for cryptodwarfs ($X_1^2 = 1.04$). The above results are consistent with those given in Table 3a (p. 24). Apparently, a dwarf background tends to oppose flowering prior to the laying down of node 13. After this time the same dwarf background tends to favour flowering.

Table 1.

The parental phenotypes and their proposed genotypes, the crosses made and the predicted F_1 and F_2 phenotypes with their expected proportions.

Line Phenotype Genotype	2 L S_1ES_2	8, 60 EI s_1ES_2	24 L S_1eS_2	53, 61 L s_1eS_2	22, 59 ED s_1Es_2	22, 58 ED s_1es_2
2 L S_1ES_2	Cross no. Parents F_1 F_2 ED:EI:L	C26 (2x8) L x EI L 0:1:3	Not made L x L L 0:0:1	C57 (2x53) L x L L 0:3:13	C53 (2x22) L x ED L 4:3:9	Not made L x ED L 16:9:39
8, 60 EI s_1ES_2			C2(8x24) EI x L L 0:3:13	C114(60x53) C126(8x53) EI x L EI 0:3:1	Not made EI x ED EI 1:3:0	C127(8x58) EI x ED EI 4:9:3
24 L S_1eS_2				C40(24x53) L x L L 0:0:1	Not made L x ED L 16:9:39	C125(24x58) L x ED L 1:0:3
53, 61 L s_1eS_2					C20(53x59) L x ED EI 4:9:3	C50(53x22) C119(61x22) L x ED L 1:0:3
22, 59 ED s_1Es_2						Not made ED x ED ED 1:0:0

Table 2.

Segregation data for gene pairs S_1/s_1 , E/e and S_2/s_2

Cross	S_1	s_1	Total	χ^2 3:1	E	e	Total	χ^2 3:1	S_2	s_2	Total	χ^2 3:1	χ^2 X:Y	% s_2
20					566	189	755	0.00	818	224	1042	6.82 ^{xx}	0.96	21.50
26	381	129	510	0.02					121	39	160	0.03	1.60	24.38
50									104	22	126	3.82	0.65	17.46
114	74	30	104	0.82	336	96	432	1.78						
119									156	36	192	4.00 ^x	0.30	18.75
125									158	34	192	5.44 ^x	0.82	17.71
126					220	45	265	9.09 ^{xx}						
127					177	56	233	0.12	233	51	284	7.51 ^{xx}	1.00	17.96
Total	455	159	614	0.26	1299	386	1685	3.93 ^x	X 1590	Y 406	1996	23.11 ^{xxx}	0.00	20.34
Exp. no.	460	154	614		1264	421	1685		1497	499	1996			
Heterogeneity χ^2				0.58				7.05					5.22	

x $P < 0.05$, xx $P < 0.01$, xxx $P < 0.001$.

Table 3.

Joint segregation data for S_1 , E and S_2 and several marker genes.

Cross	CorR	XY	Xy	xY	xy	Total	Gene X	Gene Y	χ^2 Seg.X	χ^2 Seg.Y	χ^2 Joint Seg.	RCV	\pm	SE
26	C	362	24	19	105	510	A	S_1	0.13	0.02	302.33 ^{xxx}	8.79		1.32
53	C	69	7	5	23	104	A	S_1	0.21	0.82	61.54 ^{xxx}	11.61		3.38
26,53	C	431	31	24	128	614	A	S_1	0.02	0.26	363.87 ^{xxx}	9.28		1.24
114	R	61	18	28	0	107	P	E	0.08	3.82	6.16 ^x			
126	R	155	41	65	4	265	P	E	0.15	9.09 ^{xx}	8.28 ^{xx}	30.29		5.50
127	R	126	53	51	1	231	P	E	0.76	0.32	15.07 ^{xxx}	14.85		6.60
114,126,127	R	342	112	144	5	603	P	E	0.03	10.08 ^{xx}	32.57 ^{xxx}	21.69		3.85
53,119	C	196	44	64	14	318	A	S_2	0.04	7.75 ^{xx}	0.01			
53,119,125,127	C	475	112	174	31	792	I	S_2	0.33	20.37 ^{xxx}	1.61			
127	R	176	44	57	7	284	Cy_1	S_2	0.92	7.51 ^{xx}	2.76			
119	R	88	20	32	3	143	V	S_2	0.02	6.06 ^x	1.94			
119,127	R	289	69	98	18	474	P	S_2	0.07	11.16 ^{xxx}	0.92			
53,119	C	137	28	47	10	222	R	S_2	0.05	7.36 ^{xx}	0.01			

^x $P < 0.05$, ^{xx} $P < 0.01$, ^{xxx} $P < 0.001$.

Table 3a.

Data from Cross 2 F_2 in short days showing the pleiotropic effect of length gene Cy_1 on flowering node.

	Flowering node \pm SE (m)	Flowering node \pm SE (n)	Students t	Prob.
Length Flowering	Dwarf	Cryptodwarf		
L	20.75 \pm 0.14 (96)	22.32 \pm 0.31 (28)	5.13	< 0.001
EI	12.59 \pm 0.26 (17)	11.40 \pm 0.40 (5)	2.26	< 0.05

Genotypes. Length - all plants $lelecy_2^c cy_2^c$. Dwarf = Cy_1- and Cryptodwarf = $cy_1 cy_1$.

Flowering - all plants $S_2 S_2$. L = S_1-E- , S_1-ee and $s_1 s_1 ee$. EI = $s_1 s_1 E-$.

CHAPTER 4.

The results in detail analysed one cross at a time.

Cross 26. Segregation for S_1/s_1 on an ES_2 background.

Proposed scheme.

Parents	Line 8	$s_1s_1EES_2S_2$	(EI)	x	Line 2	$S_1S_1EES_2S_2$	(L)
F_1							
F_2			1 EI	:			3 L
F_3			↓ EI				

Between-class variation.

The parents, F_1 and F_2 were grown under SD's and LD's but the F_3 under SD's only. The flowering node distributions are shown in Table 4 (p. 51). The flowering node of the EI parent is not affected by photoperiod but the L parent flowers about seven nodes later under SD's. The F_1 shows close affinities with the late parent. It flowers at a slightly lower node but responds in the same way to SD treatment. The F_2 distribution is continuous and bimodal with a marked antimode which occurs at nodes 13 - 14 in both photoperiods. Plants on the late side of the antimode behave like the late parent and the F_1 and shift later under SD's. If the two F_2 distributions are cut between nodes 13 and 14 the observed numbers in each case are in close agreement with a 3 late to 1 early ratio. (See footnote Table 4). Eleven early F_2 plants from nodes 11 and 12 bred true in F_3 . F_3 progenies were grown from two late plants flowering at nodes 14 and 16 under SD's. In each case the progenies contain both early and late types. The results are therefore consistent with the segregation of a major gene, S_1 , dominant or near-dominant for flowering at a high node number. (Dominant S_2 is universally present and as expected all plants were late in terms of flowering time under SD's).

Linkage of A and S_1 .

The A gene for anthocyanin production is segregating in this cross and as shown in Table 3 (p. 23) there is a strong linkage (recombination value 9 %) between A and S_1 .

Within-class variation.

Without adequate F_3 data inferences on within-class variation are largely speculative. Transgression in the late class of the F_2 could be explained by segregation of quantitative genes. Again the somewhat earlier position of the F_1 relative to the late parent could be explained by a change in polygenic background although it could also indicate a dosage effect for S_1 .

Cross 20. Segregation of E/e and S_2/s_2 on an s_1 background.

Proposed scheme.

Parents	Line 59	$s_1s_1EES_2s_2$	(ED)	x	Line 53	$s_1s_1eeS_2S_2$	(L)
F_1		$s_1s_1EeS_2s_2$	(EI)				
F_2 genotype (all s_1s_1)	proportion	phenotype	proportion	F_3	phenotypes		F_4
EES_2S_2	1/16	EI	9/16	EI			
EeS_2S_2	2/16	EI		3 EI : 1 L			
EES_2s_2	2/16	EI		1 ED : 3 EI			
EeS_2s_2	4/16	EI		4 ED : 9 EI : 3 L			
eeS_2S_2	1/16	L	3/16	L			
eeS_2s_2	2/16	L		1 ED : 3 L			
EES_2s_2	1/16	ED	4/16	ED			ED
Ees_2s_2	2/16	ED		ED			
ees_2s_2	1/16	ED		ED			

Major gene segregation.

The above scheme was tested through to F_4 using SD conditions. Flowering node distributions for the parents, F_1 and F_2 are shown in Table 5 (p. 52). EI plants first appeared in F_1 and all three phenotypic classes occurred in F_2 with numbers in good agreement with expectation. The discrete nature of the three classes is illustrated in Fig. 2 (p. 50) where flowering node is plotted against flowering time for F_3 plants from doubly heterozygous F_2 plants. The time and node distributions are both bimodal and discontinuous. If new axes are placed parallel to the side axes and having their origin within the minimum frequency region of each distribution the three classes ED, EI and L will fall each within a single quadrant. Three quadrants permit seven different patterns (3 singles, 3 doubles and 1 triple). Using selfed progenies these patterns will distinguish 7 of the 9 F_2 genotypes, e.g., the triple identifies EeS_2s_2 . The three ED genotypes give the same pattern and cannot be distinguished without laborious outcrossing. Progenies of fifteen seeds ($\frac{3}{4}^{15} = 0.013$) were grown from two F_2 families containing in all 119 plants. Genotypic numbers (Table 6, p. 52) are in good agreement with expectation. Two EI plants in the F_2 proved to be impenetrant eeS_2s_2 plants.

As mentioned above flowering time and flowering node distributions for F_3 descended from double heterozygotes are given in Fig. 2. The observed numbers differ significantly from the expected 4 ED : 9 EI : 3 L ratio. ($\chi^2_2 = 8.87^x$). Segregation of the E/e pair, which can be followed in the presence of dominant S_2 from the comparison EI/L, is normal ($\chi^2_1 = 0.04$). Segregation of the S_2/s_2 pair, which is obtained from the comparison L+EI/ED, is significantly disturbed ($\chi^2_1 = 8.83^{xx}$). A deficiency of recessives and hence a shortage of ED plants proved to be a regular feature of segregation for the S_2/s_2 pair of alleles (see Table 2, p. 22). Data for progenies from F_2 plants of genotypes EeS_2S_2 and EES_2s_2 are given in Table 7 (p. 53). Flowering node and flowering time

distributions are again discontinuous with minimum gaps of 4 nodes and 14 days respectively. The observed numbers are in good agreement with expectation. Progenies from F_2 plants of genotype eeS_2s_2 included 4 EI plants. The expected ratio is 1 ED : 3 L. F_4 data (lower part of Table 7) revealed these EI plants as impenetrant L plants. The adjusted segregation data are in good agreement with expectation. All ED segregates bred true in F_4 .

Within-class variation as influenced by new combinations and dosages of the major genes.

Flowering node. In terms of flowering node ED and EI plants form a single class. However ED plants flower at a lower node on the average than EI plants (see Tables 5 and 6). Even the earliest EI sub-group (Table 6 genotype EES_2S_2) is significantly later than the ED plants (difference 1.14 ± 0.30 nodes, $t_{37} = 3.88^{xxx}$). The dominant gene S_2 therefore causes a slight increase in flowering node even in the presence of dominant E. In contrast it seems likely from the close similarity between the flowering node of Line 59 (genotype EES_2s_2 , mean 9.75 ± 0.13 nodes) and the flowering node of the ED segregates (genotypes $E-s_2s_2$ and ees_2s_2 , mean 9.63 ± 0.10 nodes) that dominant E on its own has no effect. Proof of this point would involve laborious genotyping of ED plants.

Within the EI group gene E shows a dosage effect which is revealed by the comparisons EES_2S_2/EeS_2S_2 (difference 2.04 ± 0.40 nodes, $t_{24} = 5.10^{xxx}$) and EES_2s_2/EeS_2s_2 (difference 1.12 ± 0.33 nodes, $t_{39} = 3.41^{xx}$). Two doses of the E allele caused flowering at a lower node than one dose in the presence of either one or two doses of S_2 . On the other hand gene S_2 shows no dosage effect in the EI group as seen from the comparisons EES_2S_2/EES_2s_2 (difference 0.29 ± 0.37 nodes, $t_{22} = 0.78$) and EeS_2S_2/EeS_2s_2 (difference 0.63 ± 0.36 nodes, $t_{41} = 1.75$). However, gene S_2 did show a dosage effect in the L class as revealed by the comparison eeS_2S_2/eeS_2s_2 (difference 2.10 ± 0.62 nodes, $t_{18} = 3.39^{xx}$; impenetrant plants excluded).

Flowering time. EI and L plants form a single class in terms of flowering time. However, in Fig. 2 (p. 22) the EI plants are significantly earlier than L plants (difference 3.87 ± 0.45 days, $t_{312} = 8.54^{xxx}$). The third and fourth rows of Table 7 illustrate the same phenomenon. The delaying effect which dominant S_2 exerts on flowering time is therefore slightly reduced in SD's by the presence of dominant E. Under LD's of course dominant E causes a massive reduction in flowering time (compare L53 s_1eS_2 and L8 s_1ES_2 in Fig. 1 p. 12).

Some further remarks on the phenotypic differences between the classes.

The formal definition of classes ED, EI and L calls for the recording of flowering node and flowering time. The discreteness of the classes in terms of these criteria has already been discussed with reference to Fig. 2. In fact the three classes were so obviously distinct in Cross 20 that they were virtually distinguishable at a glance. In ED plants the first one or two flower buds developed into flowers and set seed whereupon further growth promptly ceased. With EI plants the early buds failed to develop into flowers and vigorous growth of the plant continued. EI and L plants also showed a marked tendency to keep growing after seed-set commenced. Consequently, these plants had a much larger number of internodes with a commensurate increase in total height and a substantial increase in yield. At maturity ED plants were very conspicuous on account of their small size (about one fifth that of L plants) and yield (about one sixth that of L plants see footnote Table 5 p. 52). The conspicuous difference between EI and L plants was the presence of undeveloped flower buds in EI plants at nodes which were vegetative in L plants.

In Fig. 2, (p.50) 3 plants flower at a slightly later time than the main ED group. These plants are closest to the ED group on formal criteria and their ED nature was confirmed visually. The slightly later flowering time of these plants was caused by abortion of the first flower buds. This may happen

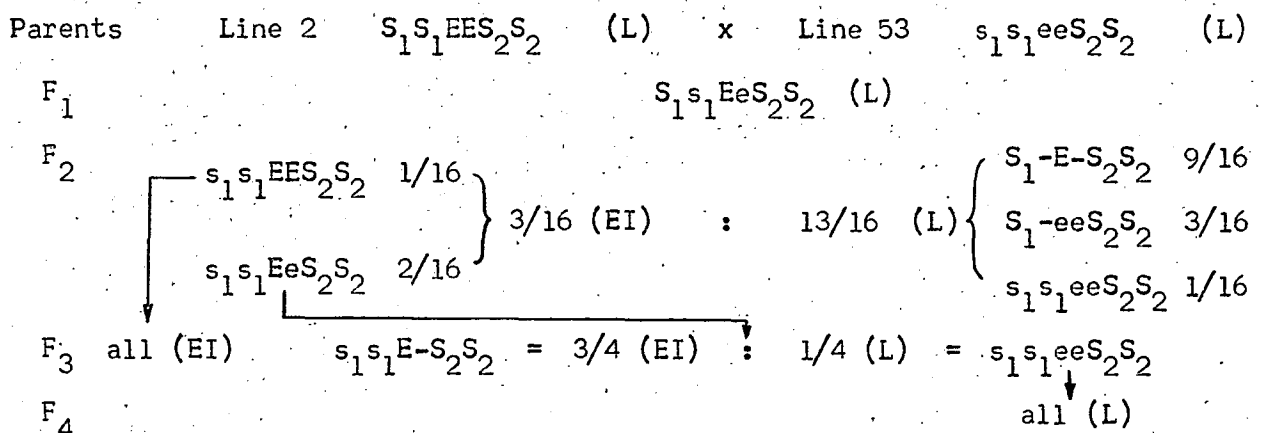
to ED plants under SD's if light conditions are poor but even under these conditions the buds usually develop to a greater extent than the lower flower buds of EI plants.

The economics of labour, time and space.

In practise sufficient separation between the classes was obtained by holding the plants in SD's only until the 15th or 16th leaf unfolded and then transferring the plants to natural photoperiod (or natural plus supplementary light). In this way SD space was freed and experimental time saved by providing conditions more favourable for seed-set and maturation. Small samples were taken through to open flower in SD's to check on extreme segregants. Visual identification of ED and EI plants along the lines described in the preceeding paragraph saves the labour of recording flowering time. These classes were visually distinguished in the F_2 of Cross 20 and no error in identification was found in the 97 plants checked out in F_3 using formal criteria.

Cross 57. Segregation of S_1/s_1 and E/e on an S_2 background.

Proposed scheme.



The genes A and S_1 are segregating in coupling and their linkage of about 10 % should cause the majority of EI segregates to have white flowers. As

indicated F_3 L segregates from F_2 EI plants should have genotype $s_1s_1eeS_2S_2$ and breed true in F_4 . However in Cross 20 a few plants of this genotype (3 - 4 %) flowered at a low node. F_5 data should reveal the true nature of any such impenetrant plants appearing in F_4 . (The observed results are in agreement with these proposals but in contrast to Cross 20 genotype $s_1s_1eeS_2S_2$ frequently flowered in the EI region misclassification rising to over 50 % in some progenies.)

Segregation of the major genes.

Two batches of F_2 plants were grown. The first batch yielded so poorly that the genotyping of EI plants could not be proceeded with. Data for the first planting are given in the top of Table 8 (p.54). Parents and F_1 were grown under both LD's and SD's but only the SD data are shown. The parents and the F_1 are each late with the F_1 intermediate between the two parents in both photoperiods. The distribution of flowering node in the F_2 under LD's is bimodal, the early mode corresponding to the standard EI region (the region characteristic of the standard EI variety L60 - node 11) and the late mode corresponding to the F_1 region. The minimum frequency class is at node 13. The distribution in SD's again has two conspicuous modes. The early mode and the minimum frequency class are in the same place as they were in the LD distribution. However, the late mode has shifted 7 nodes later in SD's but it again coincides with the F_1 mode. Approximately the same percentage of plants occurs in the first hump in both photoperiods. Genetic recombination has therefore created from two late parents sensitive to photoperiod, a class of plants whose flowering node is unaffected by photoperiod. All F_2 plants flowered late in terms of time under SD's so these plants flowering at a low node are EI-type and in fact the standard EI variety, Line 60 (s_1ES_2), is descended from a pure breeding EI plant in the F_2 of this cross. Cutting both distributions between nodes 13 and 14 gives 66 EI and 204 L plants where the expected numbers are 50 EI and 220 L. The excess of EI plants is

significant at the 5 % level ($\chi^2_1 = 6.28^x$). Yields from F_2 plants were mostly small but a few of the larger progenies were grown. As expected some EI plants bred true and some segregated, 3 EI : 1 L, but in addition, some EI plants were clearly impenetrant L-types. One F_2 plant flowering at node 11 under SD's gave in F_3 2 EI and 11 L plants, all eleven flowering higher than node 22. It is therefore likely that impenetrant L-types account for the excess of EI plants in the F_2 .

The second F_2 with its associated controls, F_1 and higher generations, is shown in the lower part of Table 8. The F_2 distribution is again bimodal but unlike the first LD F_2 it is this time continuous. In order to genotype EI plants and distinguish between genetically EI and genetically L plants in the region of overlap, progenies of 15 plants were grown under SD's from all plants flowering at node 13 or below and from a sample of plants flowering at node 14 or above. The observed numbers of 45 EI and 147 L plants, determined by this means, are acceptable as a 3 : 13 ratio ($\chi^2_1 = 3.05$) and within the EI class the 19 $s_1s_1EES_2S_2$ and 26 $s_1s_1EeS_2S_2$ plants are in reasonable agreement with a 1 : 2 ratio ($\chi^2_1 = 1.60$). However, these figures may be somewhat biased by the marked tendency in this cross for impenetrant plants of genotype $s_1s_1eeS_2S_2$ to flower in the EI region. For example genotype $s_1s_1EeS_2S_2$ has an increased chance of being classified $s_1s_1EES_2S_2$. As predicted the red/white flower colour difference did not assort independently of the L/EI difference. The strong linkage between A and S_1 is reflected in the very high value (66.7^{xxx}) of the joint segregation χ^2 .

The heterozygous EI plants generated a bimodal F_3 distribution with minimum frequency classes at nodes 17 to 19. If the distribution is cut between nodes 16 and 17 the observed numbers of 330 EI and 80 L plants show a significant deficiency of L plants ($\chi^2_1 = 6.59^x$) which is almost certainly due to the marked tendency of genotype $s_1s_1eeS_2S_2$ to flower in the EI region. This tendency is illustrated by F_4 , 5 and 6 data.

Polygenic modification of penetrance.

The F_3 L plants of genotype $s_1s_1eeS_2S_2$ are pure for the major genes and should breed true in F_4 . In fact they gave a discontinuous bimodal distribution with zero frequency at nodes 17 and 18 and about 14 % of the plants in the lower class (Table 8). F_5 and F_6 distributions descended from this F_4 all have the same distribution form as the F_4 and it is only the proportion of plants in the upper and lower classes which varies in any particular progeny or group of progenies. Eight EI plants from the F_4 gave 47 EI and 44 L plants in F_5 and two L plants gave 12 EI and 28 L plants (Table 8). The two F_5 groups have significantly different EI/L ratios ($\chi^2_1 = 5.23^x$) suggesting a significant response to selection for genes modifying the penetrance of S_2 in terms of flowering node. The F_6 data in Table 9 (p. 55) further illustrate these remarks. The data are listed as 'Line 61' grown with F_3 . Genuine Line 61 is a single plant selection from one F_4 plant of genotype $s_1s_1eeS_2S_2$ but similar pedigrees are shown as 'Line 61' for convenience.

In other crosses involving Line 53, such as Crosses 20 and 50, misclassification is rare. Line 2 seems to be the parent which supplied polygenes acting in the same direction as the dominant major gene E. It is of interest that Line 53 is a P.sativum type and Line 2 a P.arvense type.

Discussion of factors influencing flowering node in $s_1s_1eeS_2$ - plants is continued in the next cross (Cross 119/121) where the S_2/s_2 pair of alleles are segregating in the absence of major dominants S_1 and E and any EI plants must be genetically L-type.

Crosses 119 and 121. Segregation of S_2/s_2 on an s_1e background.

Proposed scheme.

Parents	'Line' 22	$s_1s_1ees_2s_2$	(ED)	x	Line 61	$s_1s_1eeS_2S_2$	(L)
F_1							
F_2			1 ED	:		3 L	
F_3			↓ all ED				

Introduction.

The first three crosses (26, 20 and 57) do not eliminate the possibility that S_1 and S_2 are alleles. One way of removing this possibility would be to show free recombination between A and S_2 , as S_1 was shown in Cross 26 to be closely linked to A . The genotype $A-s_1s_1eeS_2S_2$ was available from the F_4 of Cross 57 and the genotype $aas_1s_1ees_2s_2$ existed in the heterogeneous Line 22 along with plants carrying E . Accordingly Cross 119b was made by using the red flowered F_4 plant number 57b/3/81/8/1 as a male with a single Line 22 plant as a female. Cross 121a involved F_4 plant 57b/3/81/2/1 and again a single Line 22 plant. (There were no selfed seeds from the Line 22 mothers). The 12 F_1 plants in each case were fully late but segregated for flower colour. The red F_1 plants must have genotype $Aas_1s_1eeS_2s_2$ and were used to give the F_2 .

Segregation for A and S_2 .

The two crosses gave similar results for the segregation of A and S_2 and the combined flowering data are given in Table 9 (p. 55). The continuous distribution of flowering node in F_2 has a very un-Mendelian appearance but the segregation of S_2 could confidently be followed by observing flowering time (see last row of Table 9). Treating the EI plants as impenetrant L plants the observed F_2 numbers of 36 ED plants and 156 L plants deviate slightly from the expected numbers of 48 and 144 ($\chi^2 = 4.0^x$) but a deficiency of recessive S_2 plants is shown by the overall data to be a characteristic of segregation at the S_2 locus (see Table 2, p. 22). As predicted all ED plants bred true in F_3 .

Joint segregation data for S_2 and various markers including \underline{A} are given in Table 3 (p. 23). The RCV for \underline{A} and S_2 is 50.7 % with a joint segregation χ^2 of 0.01. There is therefore no evidence whatsoever of linkage between \underline{A} and S_2 . Also there is no evidence of linkage between S_2 and the other segregating markers, \underline{I} , \underline{V} , \underline{P} and \underline{R} . A sample of 22 heterozygous F_2 plants gave rise in F_3 to 59 plants without S_2 and 202 with S_2 . These observed numbers are close to the expected numbers of 65 and 196 ($\chi^2_1 = 0.81$) but the deviation is again towards a deficiency of recessives. The flowering time distribution for this F_3 is shown at the bottom of Table 9. Plants of genotype $s_1s_1ees_2s_2$ have flowered on the average about 4 weeks earlier than plants with genotype $s_1s_1eeS_2-$ and there is a clear gap of 12 days between the two groups. These data confirm the overall theory and show that S_2 is fully penetrant in terms of flowering time under SD's. Attention may now be given to the widespread occurrence of EI plants where none is expected if S_2 was fully penetrant in terms of flowering node.

Factors influencing the penetrance of genotype $s_1s_1eeS_2-$.

Genetic influence. The distribution of flowering node for the late parents has been discussed under Cross 57 F_5 and F_6 . The same type of bimodal distribution with minimum or zero frequency at nodes 17, 18 and 19 is shown by genotype $s_1s_1eeS_2-$ in the F_2 and F_3 of the present cross. A possible explanation of this bimodality is given at the end of Chapter 6 (p. 83). Progenies of 12 seeds were grown from all 36 EI plants flowering below node 17 in the F_2 . Fourteen* progenies contained no EI plants and of the remaining 22 only 2 consisted of more than half EI plants. The genetically late nature of the F_2 plants is therefore strongly indicated. Of these 36 F_2 plants 14* proved to be $s_1s_1eeS_2S_2$ and 22 were $s_1s_1eeS_2s_2$. The homozygotes flowered at a mean of 14.21 ± 0.41 nodes and the heterozygotes at a mean of 14.18 ± 0.37 . These figures show firstly that as approximately one third of the plants are homozygotes, misclassification

* not the same 14 plants.

is random in relation to the dosage of S_2 and secondly that as the homozygotes and heterozygotes flower at the same mean node, dosage of S_2 does not influence the actual flowering node in EI plants. These findings are supported by the F_3 data itself. The misclassification rate is closely similar whether the progeny comes from homozygous or heterozygous parents:- 22 EI to 145 L and 31 EI to 172 L respectively ($2 \times 2 \chi^2_1 = 0.33$) and the mean flowering node of the EI classes is the same to within 0.01 of a node. The mean flowering nodes of the L classes are respectively 23.88 ± 0.17 and 22.67 ± 0.18 . The difference is significant at the 0.1 % level ($t_{318} = 4.89^{xxx}$) suggesting that S_2 does show a dosage effect in L plants. These results confirm the finding from Cross 20 that S_2 shows a dosage effect in L plants but not in EI plants.

It can be seen from Table 9a (p. 56) that the male parental line used in Cross 119b has a significantly higher rate of misclassification suggesting a higher level of penetrance modifiers in this parent. (Comparison of the two parents in F_6 gives a $\chi^2_1 = 3.9^x$). This genetic potential is strongly reflected in the crosses as the misclassification rate is significantly higher in Cross 119b. (Comparison of the two F_2 s gives a $\chi^2_1 = 11.2^{xxx}$). The misclassification rate was zero in the 24 F_1 plants and the rate in the crosses is always lower than in the comparable parent suggesting that Line 22 contributed genes favouring penetrance of S_2 .

Environmental influence. It is clear from the above evidence that there is a strong genetic influence on penetrance. However, environmental factors may also exert a powerful influence. It was shown under Cross 57 by relative comparisons that selection over a single generation may significantly change the misclassification rate. Although the parental F_6 and Cross F_3 were selected from the EI class and grown under closely similar conditions to the previous generation the absolute rate of misclassification is lower. (Comparison of Cross 119b F_2 and F_3 gives a $\chi^2_1 = 15.45^{xxx}$). Presumably some small environmental

changes have more than outweighed the effect of selection. No tests have been made but it is suspected that a short period of cold treatment might trigger flowering at a low node.

Within-class variation and quantitative genes.

F_3 progenies were grown from all EI F_2 plants. The regression coefficient, b , of the F_3 progeny means (excluding ED plants) on the flowering node of the F_2 plants is -0.22 ± 0.23 which is not significantly different from zero ($t_{34} = 0.93$). The actual flowering node of plants below the threshold seems hardly to be influenced by any quantitative genes and it was shown earlier that dosage of S_2 has no influence. Presumably small environmental changes have a large influence which might be expected if the internal situation of the plants was close to the switch point between vegetative and flowering during the time nodes 10 to 16 are being laid down.

In contrast to the weak genetic influence on flowering node within the EI plants, flowering node within the ED plants is very strongly influenced by the genotype. Pure ED lines characteristically flower over a range of two nodes. The ED plants in the F_2 of Crosses 119 and 121 flowered over a range of three nodes and the F_3 derived from these, which was a much larger sample, flowered over a range of five nodes. This information leads to the suspicion that quantitative genes are segregating which are capable of shifting the flowering node slightly within the ED range. This suspicion is confirmed by the regression of F_3 progeny means on F_2 flowering node. The regression coefficient b , which is equal to the heritability h^2 , has a value of 0.38 ± 0.12 and differs significantly from zero at the 1 % level.

Cross 50. Segregation of S_2/s_2 on an s_1e background.

Proposed scheme.

Parents	'Line' 22	$s_1s_1ees_2s_2$	(ED)	x	Line 53	$s_1s_1eeS_2S_2$	(L)
F_1						L	
F_2						1 ED : 3 L	
						↓	
F_3						all ED	

'Line' 22 is recessive for s_1 and s_2 and heterogeneous for genes E and e. In this cross all gametes contributed by 'Line' 22 were of the type s_1es_2 since all F_1 plants were L-type. The results of Cross 50 are not tabled because they are closely similar to a section of the Cross 20 results already given. For example the F_1 is similar to the F_2 plants of genotype $s_1s_1eeS_2s_2$ shown in the second last line of Table 6 (p.52), the 2 impenetrant plants excluded. The F_2 and F_3 data for Cross 50 are almost identical to the F_3 and F_4 data from Cross 20 shown in the last five lines of Table 7 (p. 53). Like the F_3 of Cross 20, the F_2 of Cross 50 contains 4 phenotypically EI plants which were shown by their progeny to be impenetrant L plants. The S_2/s_2 segregation is in good agreement with expectation (see Table 2, p.22). All ED segregates bred true in F_3 . The misclassification rate of genotype $s_1s_1eeS_2-$ stands at a mere 3 % in this cross.

Development of Line 58 $s_1s_1ees_2s_2$.

Line 58, a pure line of genotype $s_1s_1ees_2s_2$, was developed by several generations of single plant selection from an ED plant in the F_2 of Cross 20.

Cross 40. Segregation of S_1/s_1 on an eS_2 background.

Proposed scheme.

Parents	Line 24	$S_1S_1eeS_2S_2$	(L)	x	Line 53	$s_1s_1eeS_2S_2$	(L)
F_1						L	
F_2						L	

The results of this cross are given in Table 10 (p. 57). As recessive e and dominant S_2 are always present all generations of the cross should flower late in terms of time and node. One quarter of the F_2 plants should have genotype $s_1s_1eeS_2S_2$ and in view of previous findings a few of these plants might misclassify as EI. The results follow the expected pattern and the few EI plants which occurred in F_2 are shown to be impenetrant L-types by the F_3 data.

Cross 2. Segregation of S_1/s_1 and E/e on an S_2 background.

Proposed scheme.

Parents	Line 8	$s_1s_1EES_2S_2$	(EI)	x	Line 24	$S_1S_1eeS_2S_2$	(L)
F_1						$S_1s_1EeS_2S_2$	(L)
F_2		$s_1s_1EES_2S_2$ 1/16	3/16 (EI)	:	13/16 (L)	$S_1-E-S_2S_2$ 9/16	
		$s_1s_1EeS_2S_2$ 2/16				$S_1-eeS_2S_2$ 3/16	
F_3	all (EI)	$s_1s_1E-S_2S_2 = 3/4$ (EI)	:	$1/4$ (L)	=	$s_1s_1eeS_2S_2$	
F_4		minority = EI (impenetrant L) + majority L					
F_5		minority = EI (impenetrant L) + majority L					

Between-class variation.

The observed data, given in Table 10 (p. 57) conform very closely to expectation. The F_1 with genotype $S_1s_1EeS_2S_2$ is fully as late as the late

parent showing that the dominant gene S_1 is completely epistatic to gene E. The observed F_2 numbers of 22 EI and 124 L plants are close to the expected numbers of 27 and 119 ($\chi^2_1 = 1.14$). F_3 progenies were grown from 9 EI plants. Three progenies, from plants flowering at nodes 10 and 11, bred true and the parental plants were probably homozygous. Six progenies, from plants flowering at nodes 12, 13 and 14, segregated. The F_2 parents in this case have genotype $s_1s_1EeS_2S_2$ which is confirmed by the near perfect F_3 figures of 37 EI and 12 L plants. As predicted the L plants gave mostly L plants in F_4 with a few phenotypically EI plants which proved in F_5 to be genetically L-type. The misclassification rate for genotype $s_1s_1EeS_2S_2$ was 8 % in the F_4 and 15 % in the F_5 but the difference in rate is not significant ($P > 0.3$).

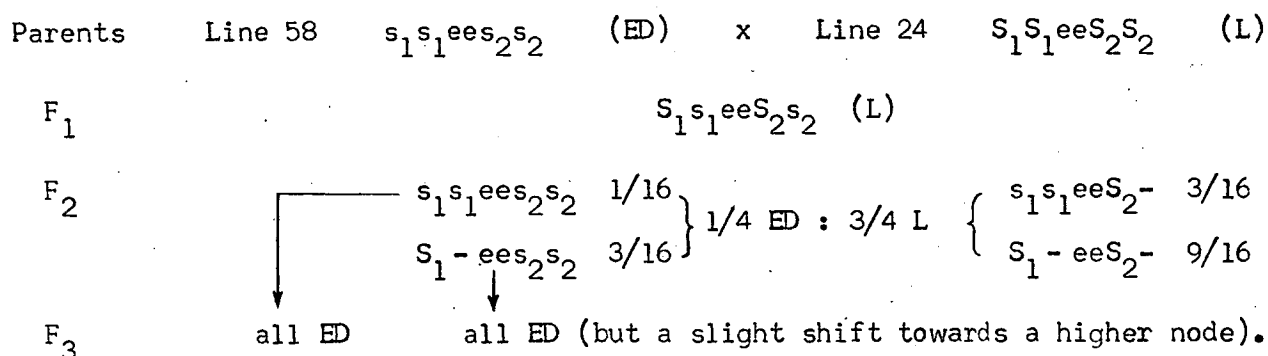
Within-class variation.

Dosage of E. The slightly higher flowering node of $s_1s_1EeS_2S_2$ as compared with $s_1s_1EES_2S_2$ found in this F_2 confirms the dosage effect for E reported in Cross 20.

Pleiotropy of Cy_1 . Line 2 is a dwarf, $le\ Cy_1\ cy_2^c$ and Line 8 is a cryptodwarf $le\ cy_1\ cy_2^c$. Consequently, the major internode length gene Cy_1 is segregating in this cross. Within the EI class dwarf segregates have a significantly higher flowering node (see Table 3a, p. 24) than the cryptodwarf segregates. Within the L class the position is reversed and the dwarfs have a significantly lower flowering node. The data suggest that Cy_1 causes changes in flowering node about one sixth the size of those associated with the major flowering genes. Although pleiotropy of Cy_1 is assumed the possibility of a minor flowering gene closely linked to Cy_1 cannot be excluded.

Cross 125. Segregation of S_1/s_1 and S_2/s_2 on an e background.

Proposed scheme.



The first occurrence of genotype $S_1-ees_2s_2$.

Cross 125 between Line 58 (s_1es_2) and Line 24 (S_1eS_2) should give an L-type F_1 . The results (Table 11, p. 58) are in agreement with this expectation. Although the genotypes of the parents were known from the results of previous crosses, the F_2 phenotypic ratio could not be predicted at the time the cross was made because a new genotype, S_1es_2 not hitherto encountered in either parents or crosses, would arise in F_2 . The expected F_2 genotypes are shown above. The observed F_2 distribution is shown in Table 11. There is a clean segregation into 34 ED and 158 L plants with a clear gap of three nodes between the classes. No EI plants occurred. If genotype S_1es_2 is an L-type, the expected phenotypic ratio in F_2 is 1 ED : 15 L. If S_1 by itself has no effect, the expected ratio is 1 ED : 3 L. Both hypotheses are consistent with previous results. The observed numbers show a very significant excess of ED plants when tested against a 1 ED : 15 L ratio ($X_1^2 = 43.02^{xxx}$) and a small but significant deficiency of ED plants when tested against a 1 ED : 3 L ratio ($X_1^2 = 5.44^x$). A slight deficiency of recessives is a consistent feature for segregation of the S_2/s_2 pair of alleles (see Table 2, p. 22). The 1 : 3 ratio is therefore clearly the most acceptable.

Maternal influence on the expression of gene S_1 .

If S_1 is not effective in the absence of S_2 the ED plants should breed true as they have done in previous crosses. The F_3 however, shows a totally

new behaviour, which was foreshadowed perhaps in the F_1 . The two F_1 samples were made by direct reciprocal crossing between single plants. Even so, the F_1 plants derived from the early mother were on the average over two nodes later than the F_1 plants from the late mother. This difference is significant at the 0.1 % level ($t_{21} = 4.64^{xxx}$). The F_2 distributions were the same no matter which way the cross was made. The effect is therefore one of maternal influence which is seen again by a comparison of the F_2 and F_3 . A large number of F_3 plants occupy a portion of the distribution which is totally vacant in F_2 . This is not the result of some change in growing conditions for a control F_2 grown with F_3 was closely similar to the first F_2 . Any genotype occurring in F_3 must also have occurred in F_2 .

Suppose:- (1) that plants of genotype $S_1-s_2s_2$ derived from mothers carrying gene S_2 flower at almost the same node as if S_1 were absent, (2) that such plants derived from mothers lacking S_2 flower a few nodes later than typical ED plants (The standard ED variety, L59, flowers at nodes 9 or 10) and (3) that in event 2 gene S_1 shows no dominance with heterozygotes more or less intermediate between the homozygous states.

Evidence on maternal influence is already dealt with above. It remains to be established that a single gene showing no dominance over its allele is responsible for the F_3 shift and that the gene responsible is S_1 .

The means of F_3 progenies are up to four nodes later than the F_2 plants. A regression of F_3 progeny means on F_2 flowering node yields the very high regression coefficient of 1.81 ± 0.16 . Coefficient b ($b = h^2$) in this case has a value over eleven times as great as its standard error. Clearly genotype played a very large part in determining the exact flowering node of the F_2 plants. Such a high heritability would be consistent with the segregation of a major gene such as S_1 . (Consider a spring compressed by maternal influence and released in F_3). A quantitative system such as the one found previously in Cross 119/121 may also have contributed to the genetic

variation in this case.

Further evidence comes from a classification of the F_3 progenies into three classes based on variance and mean, namely, low variance/low mean (2 node range, mean 10.0 - 11.5), high variance/intermediate mean (4 - 6 node range, mean 11.5 - 14.5) and low variance/high mean (3 node range, mean 12.5 - 17.0). The classes contain 8, 15 and 9 progenies respectively. These numbers correspond very closely to the 1 : 2 : 1 ratio expected if this method of classification identified respectively F_2 plants of genotype s_1s_1 , S_1s_1 and S_1S_1 . In addition the supposed heterozygous F_2 plants occur in the middle of the F_2 range (nodes 10 - 12) and the recessive and dominant F_2 plants occur at the ends of the range (nodes 9 - 10 and 11 - 13 respectively). Finally, the total F_3 distribution is trimodal with modes at 10, 12 and 16 which possibly correspond to zero, one and two doses of S_1 . If so, there is a slight tendency towards dominance of s_1 rather than a straight additive effect.

The evidence identifying S_1 as the gene responsible for the F_3 shift is more or less circumstantial. S_1 is known to be present. In previous crosses where S_1 was absent (Crosses 20, 50, 119 and 121) ED plants have shown no shift. The critical breeding tests have not been carried out as Cross 125 was made late in the crossing programme. For the time being S_1 is presumed responsible.

Future work.

Further work on genotype S_1 -ees s_2s_2 is desirable. For example the response to photoperiod is not known as all plants were grown under SD's. Under SD's, the first flower buds initiated develop into mature flowers in the manner typical of ED plants. Also in the F_3 of Cross 125 genotypes S_1 -ees s_2s_2 and s_1s_1 ees s_2s_2 show no discrete difference in either flowering node or flowering time. Genotype S_1 es s_2 is therefore classified as ED. However, this genotype was not present amongst the original parental lines and it may not fit neatly into the original phenotypic classification. For example the later S_1 -ees s_2s_2 segregates in F_3 of Cross 125 have coordinates which would place them

towards the middle of the zero frequency regions shown in Fig. 2 (p. 50).

However, in a progeny from say $S_1s_1Ees_2s_2$ or $S_1s_1EES_2s_2$ the maternal influence effect should ensure that $S_1-ees_2s_2$ (and presumably $S_1-E-s_2s_2$) segregates are clearly within the ED group. The maternal influence effect could be further checked by making the cross $S_1S_1ees_2s_2 \times S_1S_1ees_2s_2$. Direct reciprocal crosses should give very different results. Again the responsibility of S_1 for the F_3 shift could be checked by selecting plants thought to be $S_1S_1ees_2s_2$ (ED) and crossing to $s_1s_1EES_2S_2$ (EI). The F_1 (L) should be later under SD's than either parent in both flowering node and time.

Cross 53. Segregation of S_1/s_1 and S_2/s_2 on an E background.

Proposed scheme.

Parents	'Line' 22	$s_1s_1Ees_2s_2$	(ED)	x	Line 2	$S_1S_1EES_2S_2$	(L)
F_1						$S_1s_1EES_2s_2$	(L)
F_2	$s_1s_1Ees_2s_2$	1/16	$S_1-Ees_2s_2$	3/16	$s_1s_1EES_2-$	3/16	S_1-EES_2- 9/16
			ED 4/16	:	EI 3/16	:	L 9/16

Dominant genes A and S_1 in coupling.

The first appearance of genotype $S_1-Ees_2s_2$.

There are two difficulties associated with predicting the outcome of this cross. Firstly, 'Line' 22 is impure and may contribute either gamete s_1Es_2 or s_1es_2 . (This was not known at the time the cross was made). Secondly, as Line 2 has genotype $S_1S_1EES_2S_2$, plants of genotype $S_1-E-s_2s_2$ will be encountered for the first time in this cross. Assuming genotype $S_1-E-s_2s_2$ to have phenotype ED, and this is reasonable from the ED phenotype of $S_1-ees_2s_2$ plants in Cross 125, an F_1 plant of genotype $S_1s_1EES_2s_2$ should give an F_2 of

16 ED : 12 EI : 36 L and an F_1 of genotype $S_1s_1EeS_2s_2$ should give 16 ED : 9 EI : 39 L. Both F_1 plants should be L-type.

The F_2 was derived from only two F_1 plants. As the two F_2 progenies were almost identical in every respect, they are shown combined in Table 12, p. 59. As expected, the F_1 is L-type and the F_2 contains all three classes. Cutting the F_2 between nodes 16 and 17 (this cut point is more obvious on the other three F_2 distributions shown in Table 12) gives 69 red L, 5 white L, 7 red EI, 23 white EI, 16 red ED and 6 white ED. The observed numbers of 74 L and 30 EI plants differ significantly from a 13 : 3 ratio ($\chi^2_1 = 6.96^{xx}$) but are in good agreement with a 3 : 1 ratio ($\chi^2_1 = 0.82$, $P > 0.30$). The F_1 genotype is therefore taken as $S_1s_1EES_2s_2$. However, impenetrant L-types would distort a 13 : 3 ratio in the direction of a 3 : 1 and F_3 data are not available to check this point as Cross 53 plants were the last to be grown. Whether the choice of F_1 is right or wrong the assumption of phenotype ED for $S_1-E-s_2s_2$ plants is obviously supported by the data. Primary evidence comes from the fact that approximately one quarter of the F_2 plants are ED. Further evidence comes from the close linkage between \underline{A} and S_1 (approx. 10 %) known from Cross 26. The red/white ratio is obviously disturbed within the EI and L classes but is clearly undisturbed within the ED class.

Segregation details.

The S_2/s_2 segregation (Table 2, p. 22) is obtained from the comparison L+EI/ED. As usual there is a slight deficiency of recessive plants ($\chi^2_1 = 3.82$, $P \approx 0.05$). The S_1/s_1 segregation can only be followed in the presence of dominant S_2 and is obtained from the comparison L/EI. Segregation data for \underline{A} and S_1 are given in Table 3, p. 23. For both genes the individual segregation is normal but the joint segregation χ^2 is highly significant. The recombination value works out at a figure which is close to that obtained from Cross 26. The final figure from the combined data of Crosses 26 and 53 is a little over 9 %.

Cross 114. Segregation of E/e on an s_1S_2 background.

Proposed scheme.

Parents Line 60 $s_1s_1EES_2S_2$ (EI) x Line 53 $s_1s_1eeS_2S_2$ (L)

F_1

EI

F_2

3 EI : 1 L

Dominant genes E and P in repulsion.

Linkage of genes E and P.

The results are shown in Table 12, p. 59 . As expected the F_1 is EI. The distribution of flowering node in the F_2 is clearly discontinuous. The observed numbers of 349 EI and 83 L plants show a significant deficiency of L plants ($X^2_1 = 7.72^{xx}$) which, from previous experience, could be caused by some $s_1s_1eeS_2S_2$ plants flowering in the EI region. F_3 progenies were grown from 18 F_2 plants flowering at node 12 of which one plant proved to be an impenetrant L-type and from all plants flowering at nodes 13, 14 and 15 of which all but one were thought to be genotype $s_1s_1eeS_2S_2$. The corrected F_2 numbers of 336/96 are acceptable as a 3 : 1 ($X^2_1 = 1.78$). Possibly, a few more impenetrant L plants would be revealed by progeny testing the remaining EI plants. The markers A, I, P, V and R are segregating in part or all of the F_2 . (Line 60 was not fixed for all markers at the time Cross 114 was made). Gene E showed free recombination with A, I, V and R but the joint segregation X^2 for E and the sugar pod gene P is significant at the 5% level (Table 3, p. 23). Linkage between E and P is confirmed in Crosses 126 and 127.

Cross 126. Segregation of E/e on an s_1S_2 background.

Proposed scheme.

Parents	Line 8	$s_1s_1EES_2S_2$	(EI)	x	Line 53	$s_1s_1eeS_2S_2$	(L)
F_1							EI
F_2			3 EI	:		1 L	

Dominant genes E and P in repulsion.

Cross 126 is of the same type as Cross 114 and the results (Table 12, p. 59) are similar, with a deficiency of L plants in F_2 (Table 2, p. 22) and further evidence of linkage between E and P (Table 3, p. 23). Very likely the cause of the deficiency is again impenetrance of genotype $s_1s_1eeS_2S_2$ although this was not tested by growing the F_3 . However, the linkage between E and P provides evidence supporting this view. E and P are in repulsion. 9 % of L plants are genotype pp. In contrast 33 % of EI plants flowering at node 13 or less are genotype pp. However only 7 % of the 29 EI plants flowering at nodes 14 or 15 are genotype pp suggesting that many of these phenotypically EI plants may be impenetrant L plants. If these 29 EI plants are impenetrant L plants then the E/e segregation is in good agreement with expectation and the RCV for E and P works out at about 27 %. It is likely therefore that the estimate of 30 % calculated from the observed (uncorrected) data of Cross 126 is a little high. For the same reason the estimate from the combined data of Crosses 114, 126 and 127 is also probably slightly too high.

A maternal effect on flowering node is again evident from the two F_1 groups shown in Table 12. Ten plants in each group were derived by direct reciprocal crossing between single plants. Even so, the F_1 sample from the EI mother flowered 1.20 ± 0.31 nodes later than the sample from the L mother ($t_{18} = 3.88^{xx}$). No difference was found in the descendent F_2^s .

Fig. 2. Each dot represents the flowering node and flowering time for a single plant in the F_3 descended from F_2 plants of genotype $s_1s_1EeS_2s_2$ in Cross 20. The plants were grown under an 8 hour photoperiod. The expected numbers are ED (genotype $s_1s_1--s_2s_2$) 95, EI (genotype $s_1s_1E-S_2-$) 215 and L (genotype $s_1s_1eeS_2-$) 72. $\chi^2_2 = 8.49$, $P < 0.05$. The disturbed segregation results from a shortage of recessive s_2 plants which proved to be a regular feature of segregation at the S_2 locus (See Table 2).

FIGURE 2.

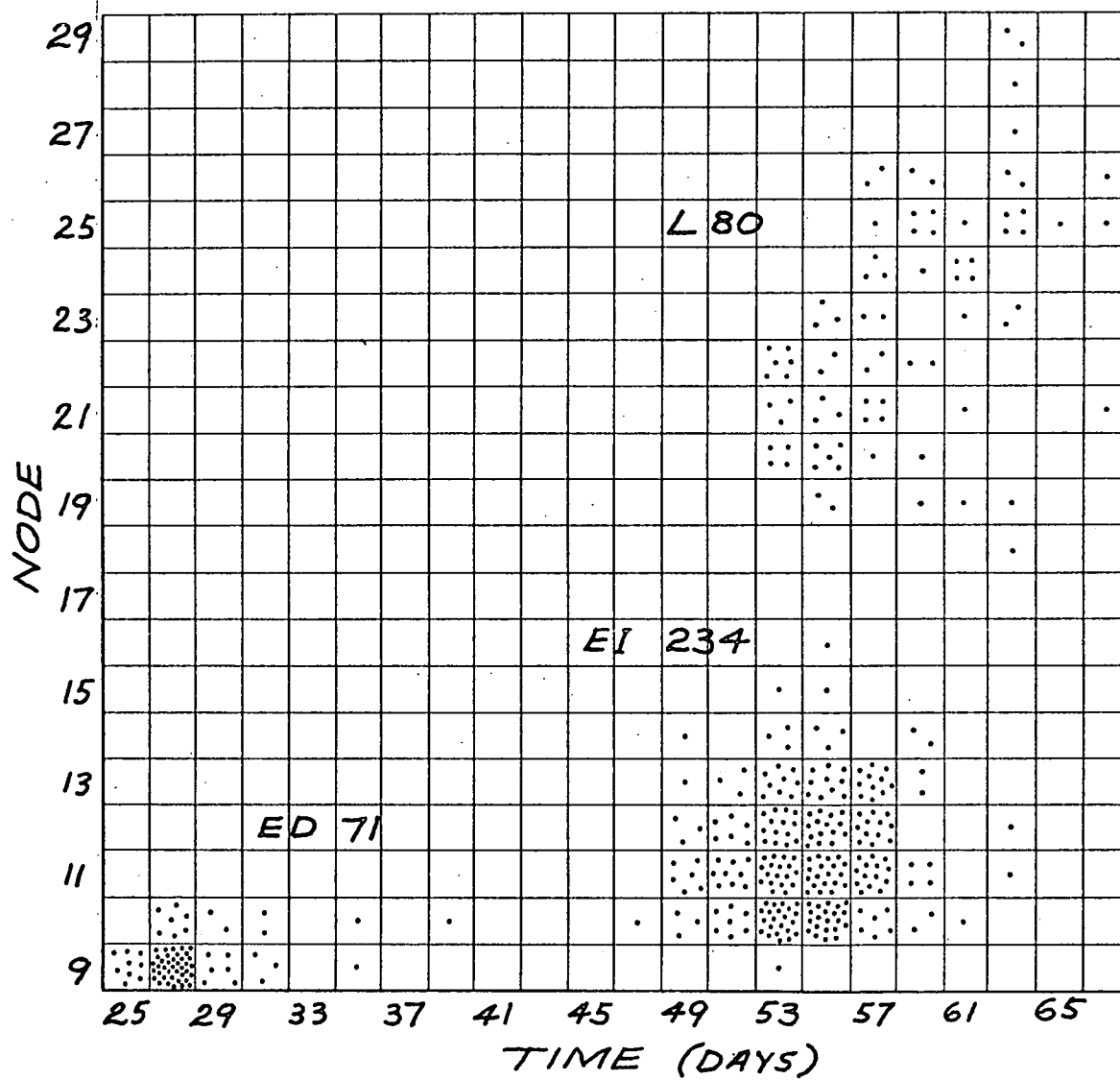


Table 4.

Cross 26. Flowering node distributions for parents, F_1 and F_2 in long days (LD = 18 hours)
and short days (SD = 8 hours) and for F_3 in SD's.

Node		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	30
Generation	Photoperiod																				
Line 8	LD	3	12	5																	
Line 8	SD	1	7	13																	
Line 2	LD								4	16	1										
Line 2	SD														1	5	4	4	3	2	
F_1	LD						5	5													
F_1	SD									1	1	0	0	4	2	1					
F_2	LD	6	18	9	2	3	38	26	21	10	1										
F_2	SD	9	50 ↓ 17	31 ↓ 4	4	3 ↓ 1	5	8 ↓ 1	3	10	11	16	37	49	37	23	28	21	19	9	3
F_3	SD	19	42	6																	
F_3	SD	11	25	6	2																
F_3	SD	1	1	0	0	1	1														
F_3	SD			1	0	0	0	1	1	0	0	0	1	0	0	0	1				

For proposed genotypes etc. see Table 1. All plants flowered at a late time under short days. $EI (s_1 s_1 EES_2 S_2) \leq$ node 13.

$L (S_1 - EES_2 S_2) \geq$ node 14. Segregation S_1/s_1 LD 99/35 = 134, $\chi^2_1 = 0.09$ and SD 282/94 = 376, $\chi^2_1 = 0$.

Combined LD + SD data for the segregation and assortment of S_1 and A are given in Table 3, top line.

Tables 5 and 6.

Table 5. Cross 20. Distribution of flowering node for parents, F_1 and F_2 . Photoperiod 10 hours.
 F_2 survival 99.7%

NODE			9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Obs. No.	Exp. Prop.	Exp. No.	χ^2
Generation	Genotype*	Phenotype																						
Line 59	EES_2s_2	ED	3	9																				
Line 53	ees_2s_2	L														2	6	4						
F_1	Ees_2s_2	EI			1	10	1	1																
F_2	$--s_2s_2$	ED	25	46	1																72	4/16	74	0.07
F_2	$E-s_2-$	EI		15	50	36	42	17	5	1											166	9/16	167	0.01
F_2	ees_2-	L										3	4	6	7	12	15	7	3	2	59	3/16	56	0.20
Total F_2			25	61	51	36	42	17	5	1	0	3	4	6	7	12	15	7	3	2	297		297	0.28
Table 6. Cross 20. Analysis [†] of genotypes in F_2 families 551/2 and 6.																								
F_2	EES_2s_2	EI		4	3	2															9	1/16	7	0.33
F_2	Ees_2s_2	EI				4	0	9	3	1											17	2/16	15	0.30
F_2	EES_2s_2	EI		3	10	1	0	1													15	2/16	15	0.00
F_2	Ees_2s_2	EI				8	9	6	2	1											26	4/16	30	0.47
F_2	$--s_2s_2$	ED	12	17	1																30	4/16	30	0.00
F_2	ees_2s_2	L														1	0	4	4	1	10	1/16	7	0.88
F_2	ees_2s_2	L					1	1						4	3	1	1	0	1		12	2/16	15	0.56
F_2	Total		12	24	26	12	16	7	2	0	0	0	0	4	4	1	5	4	2		119		119	2.54

* All plants are homozygous s_1s_1 . [†] F_2 plants were genotyped by growing, under short days, 15 F_3 seeds from each plant. The three ED genotypes cannot be distinguished by progeny testing. ED plants yielded an average of 5.9 ± 0.4 seeds, so entire progenies were grown. EI plants yielded 20.9 ± 0.8 seeds and L plants 35.7 ± 1.9 seeds.

Table 7.

Cross 20. Distributions of flowering time and flowering node for F_3 progeny from F_2 genotypes EeS_2S_2 and EES_2s_2 and flowering node data for F_3 and F_4 from F_2 of genotype eeS_2s_2 . Photoperiod 8 hours.

NODE TIME(days)				9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Obs.	Exp.	χ^2
				25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61	63-5	no.	no.	
EeS_2S_2	Node	EI	F_3		26	40	46	45	9															166	162	0.10
		L												3	7	4	6	7	6	7	8	1	1	50	54	0.30
	Time	EI														1	4	27	55	43	29	6	1	216	216	0.40
		L																	8	5	11	9	17			
EES_2s_2	Node	ED	F_3	40	7																			47	53	0.68
		EI		1	89	59	10	5																164	158	0.23
	Time	ED		28	13	4	2																	211	211	0.91
		EI														7	24	38	65	26	4					
eeS_2s_2	Node	ED	F_3	28	6																			34	37	0.24
		EI			34		2		1	1																
		L					2						6	6	26	21	11	17	11	9	3	1		115 [†]	112	0.08
ees_2s_2	Node	ED	F_4	41	89	10																		149	149	0.32
eeS_2s_2	Node	ED+L	F_4	1	8	0	0	0	0	0	0	0	0	0	1	5	4	7	4							

[†] Includes the 4 phenotypically EI plants which are revealed by F_4 as genetically L.

Table 8.

Cross 57. Flowering node distributions are given for (a) the first F_2 (SD and LD) with parents and F_1 (SD only) and (b) the second F_2 with parents and F_1 (all LD) and F_3 , F_4 and F_5 (all SD). The F_2 has been partially genotyped by growing F_3 progenies of 15 seeds.

NODE			9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29									
Generation	Genotype	Photoperiod																														
<u>First planting.</u>																																
Line 53	$s_1 s_1 e e S_2 S_2$	8											1	0	0	0	2	7	4	2												
Line 2	$S_1 S_1 E E S_2 S_2$	8																					1	3	4	9	2	1				
F ₁	$S_1 s_1 E e S_2 S_2$	8																					2	5	11	1						
F ₂		18	4	13	3	0	3	9	24	10	10	1																				
F ₂		8	3	30	12	1	4	8	2	10	5	8	6	4	11	29	23	17	13	3	2	2										
<u>Second planting.</u>																																
Line 53		18					1	2	12	5	3																					
Line 2		18							4	11	4	1																				
F ₁		18							3	8	5	1																				
F ₂	$s_1 s_1 E E S_2 S_2$	18	9	9	1																											
F ₂	$s_1 s_1 E e S_2 S_2$	18	4	19	3																											
F ₂	$\left\{ \begin{matrix} S_1 - - - S_2 S_2 \\ s_1 s_1 e e S_2 S_2 \end{matrix} \right\}$	18	19	5	5	11	31	48	30	13	2																					
F ₂	Total	18	13	33	9	11	31	48	30	13	2																					
F ₃	$s_1 s_1 E E S_2 S_2$	8	4	138	150	24	4																									
F ₃		8	1	62	129	61	49	17	6	5	1	1	0	2	6	11	18	20	9	5	5	0	2									
F ₄	$s_1 s_1 e e S_2 S_2$	8					2	1	0	3	2	0	0	2	2	16	9	14	7	1												
F ₅	$s_1 s_1 e e S_2 S_2$	8					4	15	12	6	5	5	2	1	1	3	5	12	9	8	3											
F ₅	$s_1 s_1 e e S_2 S_2$	8					1	1	1	5	4	3	2	1	1	8	10	3														

For F_6 see Table 9. Line 61 began in the F_4 of Cross 57. The Line 61 data given in Table 9 are in fact F_5 and F_6 data for Cross 57 and represent a direct continuation of Table 8.

Table 9.

Crosses 119 and 121. Flowering node distributions for parents, F_1 , F_2 and F_3 and flowering time distribution for F_3 from F_2 of genotype $s_1s_1eeS_2s_2$. Photoperiod 8 hours.

NODE GENERATION.		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	30		
Line 22		5	7																						
Line 61 grown with F ₂ (F ₅)								1	1	1	5	4	3	2	1	1	8	10	3						
Line 61 grown with F ₃ (F ₆)								3	6	5	3	5	1	1	1	5	10	15	12	4	1				
Line 61 grown with F ₃ (f ₆)										1	1	0	0	0	0	0	7	8	4	2	1				
119 + 121 F ₁																	2	5	3	2					
119 + 121 F ₂ ED EI+L		9	24	3																					
F ₂ genotype s ₁ s ₁ eeS ₂ S ₂								1	1	1	6	1	4												
F ₂ genotype s ₁ s ₁ eeS ₂ s ₂								1	3	3	6	2	7												
F ₃ genotype s ₁ s ₁ ees ₂ s ₂		1	16	165																					
F ₃ genotype s ₁ s ₁ eeS ₂ S ₂					1	1	5	4	3	3	3	2	0	0	1	12	24	36	22	23	14	6	4	3	
F ₃ from s ₁ s ₁ eeS ₂ s ₂ ED EI+L		5	30	23			1																		
TIME (days)		28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74
F ₃ from s ₁ s ₁ eeS ₂ s ₂		13	26	17	2	1	0	0	0	0	0	0	1	3	7	16	27	30	38	38	24	8	7	2	1

The 'Line' 61 data given are F_5 and F_6 data for Cross 57. F_2 plants above node 16 were not genotyped. Earlier plants were genotyped by growing 12 F_3 seeds.

Table 9a.Crosses 119b and 121a. Ratio of EI/L plants.

Generation	119b	121a	Grown together
Male parental line F ₅	9/11	6/14	1
" " " F ₆ *	19/29	4/20	2
Cross F ₂	29/46	12/68	1
" F ₃ *	48/226	5/90	2

* Descended from the EI class in the previous generation.

Table 10.

Cross 40 and Cross 2. Flowering node distributions. Photoperiod 8 hours.

<div> <div>GENERATION.</div> <div>NODE.</div> </div>	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
<u>Cross 40.</u>																			
Line 53										2	4	3	5	0	2				
Line 24														5	3	3	2	1	1
F ₁														5	5	2	2	1	1
F ₂		1	1	1	2	0	0	1	4	8	5	7	11	15	5	8	1	1	
F ₃		2	0	1	0	0	1	2	1	4	4	3	3	2					
<u>Cross 2.</u>																			
Lines 8 and 24		7	12	1								2	4	12	3				
F ₁														9	3	1			
F ₂	1	4	8	5	4	0	0	1	3	12	33	27	24	15	7	2			
F ₃ from s ₁ s ₁ EES ₂ S ₂	20	3																	
F ₃ from s ₁ s ₁ EeS ₂ S ₂	1	9	16	8	3	0	1	4	2	3	1	1							
F ₄ from s ₁ s ₁ eeS ₂ S ₂		1	1	4	0	2	3	6	6	8	12	7	9	6	6	1			
F ₅			2	1	1	0	1	1	0	4	4	2	4	2	3	2			

Table 11.

Cross 125. Flowering node distributions for parents, F₁, F₂ and F₃. Photoperiod 8 hours.

<div> <div>GENERATION.</div> <div>NODE.</div> </div>	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total	Mean	⁺ SE
Line 58	8	4																12	9.33	0.14
Line 24												2	2	3	3	2		12	22.08	0.40
F ₁ Line 58 as ♀												2	4	2	4			12	21.67	0.33
F ₁ Line 24 as ♀										2	3	4	2					11	19.55	0.31
F ₂ ED + L	3	8	15	7	1	0	0	0	1	2	6	13	24	27	40	33	12	ED 34	ED 10.85	0.16
F ₃	3	14	3	7	1													17	10.18	0.10
F ₃	1	16	15	8	4													44	10.95	0.15
F ₃	1	9	9	30	17	12	8	5										91	12.60	0.17
F ₃				3	7	10	7	14	7	1								49	14.96	0.22
F ₃								4	3	3								10	16.90	0.23
F ₃ total	2	39	27	41	28	22	15	23	10	4								211	12.82	0.16

Table 12.

Crosses 53, 114, 126 and 127. Flowering node distributions for parents, F_1 and F_2 . Photoperiod 8 hours.

<div style="display: inline-block; width: 150px; height: 40px; border: 1px solid black; position: relative;"> NODE GENERATION. </div>	8	9	10	11	12	13	14	15	16	17	18	20	22	24	26	28	30	32	34
<div style="text-align: center;">* *</div> Line 22 and Line 2		8	15										6	10	6	1			
Cross 53. F ₁ *												1	2	7	5	3			
Cross 53. F ₂ ^{ED} EI+L		1	5 1	8 4	5 8	3 9		2	4	2	1	2	3	1	13	13	11	16	11 3
Line 60 and Line 53		1	24	7								2	6	3					
Cross 114. F ₁				4	1														
Cross 114. F ₂			104	191	41	3	5	5	0	0	1	8	33	29	9	3			
Line 8 and Line 53				5	7							6	3	2					
Cross 126. F ₁ [♀] Line 8 [♂] Line 53					7 6	15 11	8												
Cross 126. F ₂			11	59	76	45	24	5	0	1	7	10	15	5	4	3			
Line 58. Line 8.		10	14 1	10															
Cross 127. F ₁				4	14														
Cross 127. F ₂ ^{ED} EI+L	1	31	15 18	4 72	53	25	5	3	1	0	12	14	15	9	1	1	3	1	

* Grown together but not with C53 F₂.

CHAPTER 5.

General discussion of the genetics.

The relationship of symbols L_f , S_n , S_1 and S_2 .

Two gene symbols, L_f and S_n are currently in use for dominant late genes in Pisum. The symbol L_f was introduced by White (1917) to replace the symbol A used by Hoshino (1915). Hoshino studied flowering time and obtained a continuous bimodal distribution with a well defined minimum frequency region. By cutting the distribution at the minimum frequency region he obtained the ratio 6 early : 10 late. He proposed a two factor hypothesis which is inconsistent, as Wellensiek (1925b) has pointed out, for if his A is a factor for late flowering time, genotype $Aabb$ should be late not early. Hoshino found linkage between late flowering and red flower colour and White states that L_f (Hoshino's A) is linked to factor A for flower colour with a RCV of 12.5 %. Lamprecht (1961) places L_f in linkage group 1, a short distance from the A locus. He defines lf as giving "First inflorescence at the 9 - 11th node; early flowering". Thus White's definition of L_f is in terms of flowering time and Lamprecht's definition in terms of flowering node.

Tedin and Tedin (1923) introduced the symbol S_n . They made several crosses between early and late lines. The distribution of flowering node was bimodal in each F_2 . The minimum frequency region was strongly defined in one case and the numbers in the early and late humps were consistent with a single factor difference with dominance of late flowering. The minima were less clearly defined in the other F_2^s but monohybrid segregations were again indicated. The Tedins attributed the segregation to a gene, S_n , "which in it's dominant state increases the number of sterile nodes below the first flower". Gene A was not segregating so there is no indication as to whether their S_n is the same as White's L_f . Barber (1959) used the symbol S_n in the same sense as the Tedins

but again there is no evidence as to whether the same gene was responsible for the segregation in both cases.

Lamprecht (1961) uses the symbol Lf but attaches to it the description of Sn which suggests that he considers Lf and Sn one and the same gene. The possibility of two or more late genes has been recognised for example by the Tedins (1923) and Gottschalk (1960). Can S_1 or S_2 be directly identified with Lf or Sn? From the linkage data it seems likely that S_1 was segregating in Hoshino's cross and that the Lf on Lamprecht's map is probably S_1 . Also it seems that S_2 is the same as the Sn gene segregating in Barber's Massey by Greenfeast cross as this cross is very probably identical to the present Cross 125. (The slight uncertainty stems from the heterogeneity of the Massey variety and the absence of F_3 data for Barber's cross). There is however no evidence to identify S_2 with the original Sn which could even be different from both S_1 and S_2 . S_1 and S_2 are defined in terms of the flowering node and time characteristics of standard varieties and their interacting roles in the three gene system. In contrast to the Lamprecht definition of Lf, S_1 does not by itself cause late flowering. The effect of S_1 is only fully manifest when substituted into genotype s_1ES_2 . Again, in contrast to the Tedin or Barber definition of Sn, S_2 does not always give a high flowering node. For example the effect on flowering node is not seen in genotype $s_1s_1E-S_2$ - or in impenetrant plants of genotype $s_1s_1eeS_2$ -.

The symbols Lf and Sn are abbreviations of descriptive terms. The symbols S_1 and S_2 are difficult to type, can be mistaken for multiple alleles and erroneously imply that the genes are duplicate factors. All things considered it would seem desirable to retain the historic symbols. I suggest that from the completion of this thesis symbols Lf and Sn replace symbols S_1 and S_2 respectively and thereafter take on the meaning which here attaches to the latter symbols. Uncertainty over gene relationships could be reduced if

new types are crossed into a pool of standard varieties. It also seems desirable to use genetically known pure varieties for physiological experiments. The purity and consistency of commercial varieties is certainly suspect in some cases (see footnote p. 93).

Over the years the crossing of pea varieties has given little evidence of a dominant early gene. A radiation mutant described by Knavel (1967) is of interest in this respect. The mutant flowered at a higher node than the Early Perfection variety from which it was derived. The F_1 tended to flower with the early parent. Knavel concluded that back mutation of sn and mutation of "another gene, gene complex or cytoplasmic factor" had occurred. If Early Perfection had genotype s_1ES_2 then a single forward mutation at E might explain his results although if his first "emerged" flower means first initiated flower, then the values for Early Perfection and the F_1 are above the usual EI range.

As regards quantitative systems the present work supports proposals put forward by Barber (1959). In particular Cross 119 F_3 demonstrates a system which operates in the absence of all three major dominants. Rowlands (1964) using 7 pea varieties has concluded that a simple polygenic system is primarily responsible for the control of flowering along with a major late gene (Sn) or effective factor. He takes the view that underlying the superficial control by a major gene (for example of photoperiod response) there is a more complex system directly determining flowering behaviour. The present work portrays the involvement of both major and minor genes but emphasises the primary role of the major genes which are shown to be responsible for most of the between-class variation and a considerable amount of genetic variation within-classes as well. It is of interest that the Cy₁ effect appears to be polygenic if flowering is the only characteristic studied. The major gene nature of Cy₁ is only revealed by the length data.

Finally, the present study does not cover all the genetic variation available in Pisum. For example, the very early Line 7, the very late Line 16 and the type with high response to photoperiod (Line 63) were not included in these crosses and may well involve further major gene differences. Also Marx (1969) has set up a phenotypic classification which is fairly similar to the one used here. His I-phenotype seems equivalent to ED and his G2-phenotype seems similar to EI. However, he has obtained a G2 F_1 by crossing two I parents. No cross between ED plants occurring in the present crosses will give rise to an EI F_1 so that a further gene is indicated.

Theoretical schemes for flowering control.

The remainder of this chapter is devoted to the exploration of speculative ideas with the object of developing a possible scheme for the action of genes S_1 , E and S_2 . Any proposals will to some extent depend on the type of model envisaged for flowering control. Flower initiation is seen as a threshold phenomenon which involves a qualitative change, with the underlying substance or substances which govern the switch varying in a continuous manner. The several schemes set out below are obviously not exhaustive but serve merely to illustrate three basic concepts, namely, control via formation of a positive stimulus, control via removal of an inhibitory substance and control in terms of an interaction between promotory and inhibitory components.

Let \emptyset stand for florigen and K stand for colysanthin (flower inhibiting substance, Barber 1959). Let F stand for flowering state and T stand for threshold level of substance. Under each scheme a minimum growth requirement may be either assumed or ignored. (The value of the ripeness to flower concept as applied to peas is questioned later in the discussion).

Scheme (1) (Promotor-only model) Flowering follows when a promotor rises above a critical level, i.e. F when $\emptyset > T$.

Scheme (2) (Inhibitor-only model) Flowering follows when an inhibitor falls below a critical level, i.e. F when $K < T'$.

Scheme (3) Flowering involves an interaction between promotor and inhibitor.

3.1 (Independent thresholds model) Absolute thresholds T and T' such that F when $\phi > T$ and $K < T'$. Inhibitor K is not required for flowering.

3.2 (Balance model) Relative threshold system whereby both substances and a favourable balance are required for flowering, i.e., F when $\phi > T > 0$ and $K > T' > 0$ and $\phi : K > \text{some constant } R$.

Further refinements and provisos may be added. For example with Scheme 3.2 there may be upper limits $\phi > T_1$ and $K > T'_1$ above which one or other substance becomes over-riding. Also R and thresholds T and T_1 may vary with age. Again we may assume that ϕ and K must also be present above certain minimum levels before differentiation of vegetative buds can proceed. This assumption makes an apparently small change to the model. However it involves a fundamental change in concept. Under 3.2 as constituted differentiation of vegetative buds may proceed in the absence of ϕ and K . Differentiation of flower buds is triggered by a favourable $\phi : K$ balance. Suppose that ϕ and K are transmitted to the apex where a favourable balance switches on a certain gene system(s) and that ϕ and K do not participate directly in morphogenesis. The assumption that minimum levels of ϕ and K are required for the differentiation of both flower and vegetative buds leads logically to the further assumption that ϕ and K participate directly in morphogenesis. In the arguments which follow it is intended that both concepts be covered by the term "balance" model.

In practice the several schemes are not readily distinguished as, in effect, a fall in the level of inhibitor is the same as a rise in the level of promotor. This point by itself justifies the inclusion of models involving an inhibitor. Selective forces select for a particular effect, which allows for

the evolution of genetic systems regulating via either promotory or inhibitory substances. A scheme using inhibitor-only, runs counter to the generally accepted views on flowering but there seems little fundamental difference between a scheme which assumes the vegetative state when K is above a critical level and one which assumes the flowering state when ϕ is above a critical level. The preoccupation with promotor stems presumably from the fact that it is the step vegetative to flowering which is usually under consideration. However, the reverse step of flowering to vegetative form also receives considerable attention in this thesis.

With the balance model control is achieved not by the presence or absence of one or other substance but by variation in the proportion of the components of the balance. Indeed ϕ and K may well be important metabolic compounds essential to the life of the plant. Such a balance model fits in with results for auxin-kinetin interaction (Sköog and Miller 1957) and with more recent findings on hormone interaction surveyed by Galston and Davies (1969). Further the balance model would seem to provide flexibility in that there is a dual opportunity for genetic regulation and environmental influence, yet stability in that a factor which proportionately lowered both components would have no effect. (With the independent threshold model a change in state could occur in this way).

A speculative model of gene action.

Action of Cy_1 . With any scheme the metabolic situation at a particular point in time will depend on the total genetic constitution and the environment past and present. Because of the complex interrelationship of substances in the metabolic pool, it is assumed that changes in the level of many substances not directly involved in the flowering process may influence in a small way the flowering reaction. Some of the genes of small effect in these crosses could be acting in this indirect manner which could explain why major gene Cy_1 has a

Pleiotropic effect on flowering although it is primarily concerned with internode-length.

Action of gene S_2 . Genes with a major effect on flowering are probably concerned directly with the primary components of the flowering process. Gene S_2 may be taken as a starting point because, of the three genes, it has the most widespread effect on flowering and the life of the plant. Gene S_2 is dominant for a delay in flowering node and time. On Scheme 1 gene S_2 could be suppressing a flower promotor. However it is certainly more comfortable to picture a positive role for gene S_2 and this approach is now followed although Scheme 1 remains an alternative. Thus under Schemes 2 or 3 gene S_2 could be responsible for the production of a flower inhibitor. This inhibitor qualifies as K but specifically it will be referred to as S_2 -substance because the same substance is presumably responsible for the suppression of floral development under SD's and also the growth prolonging effect of gene S_2 .

Genotype s_1eS_2 flowers at node 9. Irrespective of the factor(s) limiting flowering prior to node 9 in s_1eS_2 plants, it follows that the vegetative state above node 8 is maintained by a high level of inhibitor. Whether the axillary bud at node 9 will be a flower bud or a vegetative bud is probably already determined by the seventh or eighth day from germination and at this early stage the plants have very little green tissue (see footnote). This suggests that the cotyledons are a source of inhibitor. However s_1eS_2 plants seldom flower below node 20 in SD's and may remain vegetative for more than 30 nodes. It seems unlikely that inhibitor contributed weeks before is still

Footnote. I have found (Murfet, unpub.) that 1 ug of gibberellic acid will delay flowering in the early variety Massey only if applied to the seed or seedling prior to the 8th day. Haupt (1952 and 1969) proposes that in the early variety Kleine Rheinlanderin, initiation of flowers at the 9th or 10th node takes place about the 5th day after soaking ~~and~~ at which time the plants are still underground.

enforcing the vegetative state at these high nodes. More likely other parts of the growing plant are capable of making and exporting inhibitor to the apex. Green tissue, which includes stipules and stem, is an obvious suspect for this role, particularly in view of the photoperiodic sensitivity of s_1eS_2 plants. Clearly the inhibitor concentration drops below the critical level at an earlier age under LD's which suggests that inhibitor production may be a dark reaction whose product is destroyed by LD's. However, even under SD conditions all plants of this genotype flowered before node 35. As the plant ages it seems to move inexorably toward the flowering state. In impenetrant s_1eS_2 plants which initiate at say node 10, the lower flower buds are completely suppressed, a situation which gives way to strong retardation by node 16 and weak retardation by node 20. This suggests a gradual fall in the level of S_2 -substance as the plant ages. Possibly, the plant loses the ability to make inhibitor or some destructive mechanism is switched on as aging proceeds.

Action of gene E. Genotypes s_1es_2 , s_1Es_2 and s_1ES_2 all initiate their first flowers in the region of nodes 9 - 12. The first two genotypes are phenotypically similar but s_1ES_2 is a very different type of plant which is phenotypically indistinguishable from the occasional impenetrant plants of genotype s_1eS_2 which initiate in the 10 - 12 region, i.e., the lower flower buds fail to develop, growth is prolonged and yield increased etc. These facts suggest that E is not producing some florigenic substance but is specifically modifying one effect of gene S_2 , namely the inhibition of flower initiation. The other effects of S_2 are not changed and it is clear that at the time nodes 10 - 15 are expanding appreciable quantities of S_2 -substance are available. Gene E is therefore not causing a general lowering of the level of S_2 -substance throughout the plant. Several possibilities exist including lowering the level of S_2 -substance at a particular site such as the cotyledons or the apex or a reduction in the sensitivity of the apex to the inhibitor. The phenomenon

of vegetative reversion in EI plants provides evidence on this point as well as further justification of the assumption that S_2 -substance is produced in both the cotyledons and the shoot.

As described in Chapter 3, 27 % of $s_1s_1E-S_2$ - plants in the F_2 of Cross 126 showed vegetative reversion for at least one node. The vegetative reversion occurred most frequently at nodes 14 or 15 in this cross but in other crosses node 16 was more common. Typical patterns were 15V, 14V-16V, or more rarely an oscillating pattern such as 13V, 15V, 17V. Nodes 14 and 17 are laid down respectively about the time the second and fourth pinnate leaves are expanding. By the time node 14 is laid down and from then on non-cotyledonary portions of the plant, in particular the leaves, must be exerting an increasing influence on the behaviour of the plant. By the time node 17 is laid down, cotyledonary influence is probably relatively minor. This evidence suggests that gene E lowers the level of S_2 -substance in the cotyledons so that at the time nodes 10, 11 and 12 are laid down, the level is below the threshold and flowering takes place. However, supplies of inhibitor from the shoot boost the level above or close to the threshold by the time nodes 14 - 17 are being laid down. It is presumably the closeness of the inhibitor level to the threshold which enables the internode-length genes in Cross 126 to bring about substantial changes in the frequency of vegetative reversion.

Before leaving gene E, a further possibility under Scheme 3.2 should be considered. From the closely similar flowering behaviour of s_1es_2 and s_1ES_2 it would appear that E is not increasing the production of a promotor. However the timing of promotor production or mobilisation in the cotyledons may be used to explain this similarity. Thus s_1ES_2 cotyledons may export increased levels of both promotor and inhibitor relative to s_1es_2 cotyledons with the balance turning in favour of the flowering state about the time the 10th or 11th node is initiated.

The action of the penetrance modifiers of S_2 . The polygenes which lower the penetrance of S_2 in terms of flowering node may operate in the same way as gene E. Line 61 (s_1eS_2), which is rich in these polygenes, gives a bimodal distribution of node of first flower under SD's. Zero or minimum frequency occurs at nodes 16 and 17. Line 53 which has the same major genes but lacks the polygenes, hardly ever flowers below node 18. Possibly Line 61 differs from Line 53 by having a lower availability of S_2 -substance in the cotyledons. The minimum frequency region in the distribution of flowering node for Line 61 corresponds closely with the region at which vegetative reversion is most likely to occur in s_1ES_2 plants. Of course s_1eS_2 plants which initiate below node 18 may also show vegetative reversion, sometimes for 8 or more nodes. Inhibitor levels in Line 61 are evidently clustered around each side of the threshold. As such this variety may represent a good test plant as treatments which normally have only a small effect may be expected to cause substantial changes in penetrance and mean flowering node. It is curious that the dose of S_2 does not effect the percentage of plants flowering earlier than node 18 (see Cross 119).

The action of gene S_1 . The behaviour of S_1Es_2 and S_1es_2 is not yet fully investigated, but these genotypes apparently flower a few nodes later than s_1Es_2 and s_1es_2 in SD's. The latening effect of S_1 almost vanishes if the seed is derived from mothers carrying S_2 . Line 24 (S_1eS_2) and Line 2 (S_1ES_2) never flower below node 17 in SD's although Line 2 carries gene E and probably an abundance of penetrance modifiers. Genotype s_1ES_2 flowers at a low node as do impenetrant s_1eS_2 plants. The major effect of S_1 is therefore to ensure the delaying action of S_2 on flower initiation although it causes a small delay in flower initiation in the absence of S_2 .

S_1 is not merely suppressing gene E as S_1es_2 plants are a few nodes later than s_1es_2 plants. Nor is S_1 a duplicate gene to S_2 as S_1es_2 plants show no evidence of suppressed floral development in SD's or prolonged growth.

Consider first the Inhibitor-only model. Suppose gene S_1 increased the sensitivity of the apex to inhibitor so that the threshold for S_2 -substance lay at a lower level. This would explain why S_1ES_2 and S_1es_2 plants invariably flower at a high node as it was earlier argued from the incidence of vegetative reversion that inhibitor levels were close to the threshold in s_1ES_2 and impenetrant s_1es_2 plants. Again the lower threshold could explain the slightly increased flowering node of S_1es_2 plants if a low level of inhibitor is assumed to be present in the early life of recessive s_2 plants. (Recessive s_2 could be a leaky mutant or possibly inhibitor is made via some other metabolic pathway). Alternatively S_1 may be a modifier specifically increasing the output of inhibitor by the S_2 locus.

The same two possibilities concerning inhibitor exist under Scheme 3.1 (Independent thresholds model) but the possibility that gene S_1 is involved with promotor also arises. Suppose gene S_1 reduced the level of promotor or raised the threshold for promotor. Genotype S_1es_2 flowers about node 14 and s_1ES_2 about node 11. These suppositions may therefore be rejected as they would erroneously predict S_1ES_2 to flower early when in fact it is fully late.

Under Scheme 3.2 (Balance model) S_1 may render a given quantity of inhibitor more effective by firstly changing the critical $\phi : K$ ratio, secondly improving transport or incorporation of inhibitor into the apex or thirdly lowering the level of promotor. In effect these proposals mean increased sensitivity to inhibitor. On the other hand gene S_1 may raise the level of inhibitor.

The same proposals therefore arise under each of Schemes 2, 3.1 and 3.2, namely, that gene S_1 , in effect, increases sensitivity to inhibitor or it increases output of inhibitor by the S_2 locus.

The suggestion that gene S_1 prolongs the juvenile phase from say 8 to 13 nodes will explain the position of S_1es_2 but it will not explain why S_1ES_2 is

fully late when only a small percentage of s_1ES_2 plants (27 % in the F_2 of Cross 126) show vegetative reversion in the 14 - 17 region. Again it does not explain why Line 2 (S_1ES_2) and Line 24 (S_1eS_2) are consistently later than Line 53 (s_1eS_2). In fact does the concept of a juvenile phase really add anything to the schemes? Presumably, a plant is vegetative during this phase because the chemical situation at the apex does not favour flowering, which means that some promotory substance(s) is inadequately present or some inhibitory substance(s) is too abundantly present or both situations apply. The question of which factor is limiting flowering in s_1es_2 plants whilst the first 8 nodes are laid down is better deferred at this point.

Finally, the maternal effect on the expression of S_1 is the reverse of what would be expected if S_2 -substance were transferred from mother to seed. It was suggested earlier that the inhibitor level seems to fall inexorably in S_2 plants as the plant ages. It is possible that in these plants a gene system becomes activated, perhaps by mere aging or by continued high concentrations of S_2 -substance, which destroys S_2 -substance or shuts down its production. The maternal effect possibly arises because the same system becomes activated in S_1es_2 seeds carried on S_2 mothers, i.e., such seeds are prematurely aged.

Conclusion.

The speculative proposals put forward here may be summarised as follows. Gene S_2 produces a flower inhibiting substance in the cotyledons and shoot. Gene E lowers the level of inhibitor in the cotyledons. Gene S_1 in effect increases apical sensitivity to inhibitor. How this effect is achieved varies with the scheme proposed but amongst other things it could involve a reduction in promotor level under the balance model. Alternatively, gene S_1 increases output of inhibitor by the S_2 locus. This model of gene action satisfies the three Schemes, 2, 3.1 and 3.2. Under each Scheme it is necessary to assume

small quantities of inhibitor in recessive s_2 plants. The simplest model occurs with the inhibitor-only Scheme 2. As well as inhibiting flower initiation, the substance produced by the S_2 gene (the S_2 -substance) also suppresses floral development and prolongs growth with the consequence that total nodes, height and yield are increased. In the broad sense S_2 -substance promotes vegetative growth and opposes flowering and senescence. The inhibitor level is reduced by long photoperiods and by the aging process itself which may operate by switching on some destructive mechanism or switching off the gene. The maternal influence which S_2 mothers exert on S_1s_2 offspring may result from premature aging.

As Schemes 1 and 2 are reciprocal arguments it follows that a model of gene action should be possible under the promotor-only scheme. Suppose the gene for promotor is universally present in the varieties used. Then, gene S_2 lowers the level of promotor in the cotyledons and shoot, gene E suppresses the action of S_2 in the cotyledons and gene S_1 decreases apical sensitivity to promotor. With this model all three genes have a regulatory function.

PART. III.

A PHYSIOLOGICAL EXPERIMENT TESTING THE PROPOSED MODEL OF GENE ACTION.

CHAPTER 6.

Intervarietal grafting with known genotypes.

Introduction.

The ideas put forward in Part II on the possible action of genes S_1 , E and S_2 have been tested here by intervarietal grafts made at an early age. Unfortunately, only 6 of the 8 pure genotypes were available but reciprocal grafts in all possible combinations have been made between these 6 lines.

Materials and Methods.

Growing medium and conditions, photoperiod control, scoring techniques, terminology and lines used are all described in Chapter 2.

The six lines are:-

Line	59	58	60	53	24	2
Genotype	s_1Es_2	s_1es_2	s_1ES_2	s_1eS_2	S_1eS_2	S_1ES_2
Phenotype	ED	ED	EI	L	L	L

As well as recording node of first initiated flower, the plants were allowed to grow on, and all nodes above the first flower were checked for vegetative reversion. Data were recorded from the main shoots only.

Grafting procedure is as follows. The seeds were set to germinate at room temperature in wet vermiculite. The grafts were made at 4 days when the plumules were still crooked and some 8 - 16 mms. long. For the stock the epicotyl was cut off just below the first scale leaf. A tiny rubber band made from bicycle valve rubber was slipped over the cut top of the stock and the epicotyl slit down the middle by a sharp scalpel. For the scion the epicotyl was cut off just above the cotyledons, cut into a wedge shape with a very sharp razor blade and wedged into the stock. The rubber band keeps the surfaces in contact till a firm union takes place. This occurs within 24 hours and good grafts are growing vigorously by a week. The cotyledonary axils were checked

at regular intervals and any lateral shoots excised. Some grafts were very slow to take. These grafts typically took 2 - 4 weeks to show appreciable growth and seldom attained a state of vigorous growth. Such grafts, referred to here as slow grafts, have been analysed separately as their flowering behaviour is often substantially different from that of their vigorous counterparts.

A few grafts failed altogether. In particular the red flowered varieties L60 and L2 made very poor stocks. There are 42 treatments - 6 ungrafted controls and 36 grafts. Early varieties like L59 initiate very soon (5 - 7 days) after germination and the aim was to start and complete the grafts on the 4th day. To meet this time requirement it was only possible to cope with six plants per treatment. The experiment was therefore carried out three times in order to get sufficient numbers, i.e., replicated in time and space. A SD photoperiod of 8 hours was employed throughout the experiment. Results for ED scions from Replicate No. 1 are not included in the analysis as the grafts were apparently made a little too late in some cases for the node of first flower to be influenced and the grafts on L2 stocks failed. Spare grafts were made to L2 stocks in the second and third replicates in order to get workable numbers.

Results.

The results given in Table 13 and Figs. 3 and 4 (pages 89-91, 86 and 88) show some very marked patterns, differences and similarities. Firstly, the distribution of mean node of first flower in Fig. 3 is clearly bimodal. Like the segregating progenies in the crossing programme there is a zero frequency region at nodes 15 - 17. Individual plants very rarely flowered for the first time in this region. Grafted scions could be classified therefore into discrete classes, early and late, on the basis of flowering node. Scions with gene S_2 are capable of appearing in both classes, e.g., scion 60 is early on

its own cotyledons but late when grafted to 53 cotyledons. The reverse effect is observed with scion 24 which is late on its own cotyledons but early when grafted to 58 cotyledons. However, in graft combinations giving the 24/58 type of result a minority of scions may fail to switch classes in response to grafting. These rare 'ineffective' grafts are not used in calculating the node of first flower. They are listed separately in Table 13 in the column with the heading "atypical grafts". This apparent qualitative difference within a single treatment falls into perspective when the status of nodes above the first flower is taken into account and is discussed more fully when grafts of the type 24/58 are considered.

Comparing the behavioural patterns of the scions, we find they form three pairs.

1. 59 s_1Es_2 and 58 s_1es_2 .
2. 60 s_1ES_2 and 53 s_1eS_2 .
3. 2 S_1ES_2 and 24 S_1eS_2 .

The flowering behaviour of the scion is independent of gene pair E/e and dependent on the genotype at the S_1 and S_2 loci.

Comparing the stocks, we find they also form three pairs.

1. 59 s_1Es_2 and 58 s_1es_2 .
2. 60 s_1ES_2 and 2 S_1ES_2 .
3. 24 S_1eS_2 and 53 s_1eS_2 .

The behaviour of the stock is independent of gene pair S_1/s_1 and dependent on the genotype at the S_2 locus and also the content at the E locus when dominant S_2 is present.

It is already apparent from a quick appraisal of the results that they show general agreement with the proposals of gene action deduced in Chapter 5 from the crossing results. As expected S_2 is active in both cotyledons and

shoot and E seems active only in the cotyledons. In addition S_1 seems only active in the shoot and this finding fits well with the proposal that S_1 increases apical sensitivity to inhibitor whilst it removes the alternative proposal that S_1 increases output of inhibitor by the S_2 locus as this model would erroneously predict S_1ES_2 to flower early and subsequently revert to the vegetative state. The graft results are now examined in detail in relation to the proposals that gene S_2 produces inhibitor in the cotyledons and shoot, gene E lowers the level of inhibitor in the cotyledons and gene S_1 , in effect, increases apical sensitivity to inhibitor. These proposals are valid under Schemes 2, 3.1 and 3.2. The results for vigorous grafts may all be explained solely in terms of inhibitor level and threshold. As it is usual to proceed with the simplest hypothesis, Scheme 2 is used until such time as it proves inadequate.

Self - grafts. The self grafts are the true controls in this experiment. They are in all cases later than the ungrafted controls. In some cases the difference is statistically significant but it is always small in both relative and absolute terms. Certainly, normally growing scions never change classes when self-grafted and within-class changes caused by self-grafting are small compared with some of the changes observed with intervarietal grafts. For example 58/58 is 0.82 ± 0.20 nodes later than the 58 controls ($t_{20} = 4.15^{xxx}$) but 58/53 is 3.62 ± 0.31 nodes later than the 58 controls with t_{20} equal to 11.68^{xxx} . Where a self-graft fails to grow normally substantial changes may occur in the scion but this phenomenon is dealt with under the heading of slow grafts.

Grafts using scions of genotype s_1s_2 . The grafts were performed on the 4th and 5th days from the commencement of germination. At this time, scions of 58 (s_1es_2) and 59 (s_1Es_2) were very close to initiating their first flower. According to the model these scions, which lack gene S_2 , should be almost incapable of producing inhibitor themselves. This prediction is born out by

the fact that they are never made late by any stock. With gene s_1 they are expected to have low sensitivity to inhibitor. Any delay which they show will reflect the level of inhibitor contributed by the stock cotyledons. Both scions show an identical pattern of response to the grafts. 58 scions are almost invariably about 0.8 of a node later than the comparable 59 scions. It could be that gene E is operating in the scion but the uniform size of the difference suggests that it is caused by some property of the scion tissue which does not involve the inhibitor itself.

According to the model cotyledons with gene S_2 will be rich in inhibitor with gene E lowering the inhibitor level when present. In relative order of delaying power, we might expect the series $59 \leq 58 < 60 = 2 < 24 = 53$. The observed results are in excellent agreement with this pattern although Line 2 cotyledons seem to have a little more delaying power than expected. As befits the low sensitivity of s_1 scions the delays induced by the S_2 stocks are not large in absolute terms. (Inhibitor presumably breaks down or is used up or destroyed within a limited time). However, comparisons between stocks 59 and 60 or 60 and 24 are statistically significant at the 1 % level for scion 59 and the 0.1 % level for scion 58. eS_2 cotyledons are only capable of delaying s_1s_2 scions to node 13. It is clear therefore from the high flowering node of s_1S_2 scions grafted to eS_2 cotyledons that inhibitor is produced in the shoot as well as the cotyledons (compare grafts 58/53 and 53/53) which supports the same proposal put forward previously on the evidence of vegetative reversion. Vegetative reversion was extremely rare in ungrafted ED plants. Grafted s_1s_2 scions sometimes showed vegetative reversion. This usually occurred at the node immediately above the first flower and continued for no more than 2 nodes. A typical example from treatment 59/24 would be 10 F, 11 V, 12 F - ∞ . ED scions are on the point of laying down the first flower primordium at the time they are grafted, so it is presumed that the first flower was initiated before the inhibitor from the donor cotyledons rose above threshold level.

Vegetative reversion in s_1s_2 scions is therefore caused by inhibitor from the stock cotyledons. (Compare with S_2 scions where vegetative reversion occurs at a higher node and is caused by increased levels of inhibitor being supplied by the scion itself).

S_1S_2 scions 2 and 24 on stocks 59 and 58. Over the four grafts 2/59, 2/58, 24/59 and 24/58 there were 5 slow grafts and 56 vigorous grafts. Slow grafts are discussed later. Of the 56 vigorous grafts, 11 flowered permanently from an early node, 36 flowered temporarily at an early node and 9 flowered at a late node. Controls and self-grafts of Lines 2 and 24 flowered at a high node. The most significant features of these results with ED stocks are (1) the high percentage of these late scions which are caused to initiate their first flower at a low node and (2) the transient nature of this first flowering. Presumably, the late scion contains very little inhibitor when it is removed on the 4th day from its own inhibitor-rich stock. It is then grafted to a new stock almost free of inhibitor. While the scion is small the inhibitor level is low. Flowering follows about node 12 or 13. As the cotyledons are shrinking, shoot tissue is increasing and because the shoot contains S_2 and is under SD's increasing quantities of inhibitor enter the system, the threshold is exceeded and vegetative reversion results. Over 75% of the scions which were caused to flower at an early node, reverted to vegetative after flowering for 2 - 4 nodes and remained vegetative for 6 - 8 nodes before commencing to flower for the second time. The second flowering in these grafted scions is thought to follow, like the first flowering in control plants, from an inevitable fall in the inhibitor level as the plant ages. This question of aging has already been dealt with in Chapter 5. Grafted scions usually commenced stable flowering (their second flowering) about 4 - 6 nodes earlier than the control grafts commenced stable flowering (their first flowering). Possibly the presence of flowers at a few early nodes has precipitated an earlier onset of the aging process. However, it may simply be that a lower total amount of inhibitor

entered the system as grafts which failed to flower at an early node (see atypical 24/59 and 24/58 plants, Table 13) also commenced stable flowering at a lower node than the controls.

With one exception all scions which flowered permanently from an early node occurred in the first replicate of the experiment and all scions which failed to flower at an early node occurred in the second and third replicates. The same procedures were used in every case but slight differences in time of grafting and environmental conditions have caused what are apparently qualitative differences in behaviour. The differences are qualitative only because of the threshold nature of the phenomenon. The underlying cause is considered to be a continuously varying level of inhibitor determined by an interaction between genes major and minor and the environment. Further these two types are only the extreme ends of a gradational series. The penultimate members of the series are seen in graft 2/59. One plant flowered at node 14 and reverted to vegetative at only one node thereafter. Another plant flowered at node 13 and reverted to vegetative at nodes 14 through 25.

s_1S_2 scions 60 and 53 on stocks 59 and 58. Grafts of s_1S_2 scions 60 and 53 on stocks 59 and 58 also resulted in 56 vigorous grafts. Of these 50 flowered permanently from an early node (cf 11 in the case of S_1S_2 scions), 6 flowered temporarily at an early node (cf 36 for S_1S_2 scions) and not one scion (cf 9 for S_1S_2 scions) flowered at the late node characteristic of scions 60 and 53 grafted to 53 cotyledons. The lower sensitivity of s_1 scions to inhibitor is clearly evident from the comparative figures for the S_1S_2 scions. In addition although S_1S_2 and s_1S_2 scions are both caused to flower at an early node by grafting to s_2 cotyledons, s_1S_2 scions flower first at almost the same node as the s_1s_2 scions (compare for example 60/58 or 53/58 with 58/58) whereas the S_1S_2 scions flower first at nodes 12 to 14 which is some 2 to 4 nodes later (compare for example 24/58 or 2/58 with 58/58). It is

regrettable that a pure S_1es_2 line was not available for grafting but it may be noted that the flowering node of 24/58 ties in closely with the flowering node of S_1es_2 plants in the F_2 of Cross 125 (24 x 58). It seems that the first flowering node of s_1S_2 or S_1S_2 scions when grafted to s_1es_2 cotyledons is independent of the presence of S_2 but depends on the genotype at S_1 . This behaviour also reflects the greater sensitivity of S_1 apices to inhibitor which was presumably supplied in small quantities by the s_1s_2 cotyledons.

S_1S_2 scions 2 and 24 on stocks 60 and 2. Scions 2 and 24 are invariably late when grafted to 60 or 2 cotyledons. Presumably, this occurs because the S_1 scions have a high sensitivity to inhibitor and the moderate levels of inhibitor supplied by ES_2 cotyledons are sufficient to maintain the inhibitor level above the threshold until the supply from the S_2 scion itself reaches an effective level.

s_1S_2 scions 60 and 53 on stocks 60 and 2. Three of the four graft types, 60/60 and 60/2 and 53/60 flower at an early node. Presumably the moderate supply of inhibitor from ES_2 cotyledons does not push inhibitor levels above the high threshold obtaining in s_1 apices. Graft 53/2 is somewhat of an exception. Of the 12 vigorous grafts only 1 flowered at an early node. The other 11 flowered at the late node characteristic of 53 controls. This behaviour is thought to result from a combination of two circumstances. Firstly, grafts with scions 59 and 58 show that 2 cotyledons probably have a little more inhibitor than 60 cotyledons. Secondly, a comparison of scions 60 and 53 on eS_2 stocks suggests that 53 scions produce more inhibitor than 60 scions.

Scions S_1S_2 and s_1S_2 on stocks 24 and 53. Scions of 60, 53, 24 and 2 are invariably late when grafted to stocks of 24 or 53. These inhibitor-rich eS_2 stocks donate sufficient inhibitor to keep even the low sensitivity s_1S_2 apices vegetative until inhibitor formed in the S_2 scions is abundant enough to maintain the vegetative state. This results in a change of class for 60 scions which are early on their own cotyledons. Although all 4 scions fell within a single class there are substantial differences between scions. S_1 scions

are always later than s_1 scions. This would be expected with the greater sensitivity of S_1 scions which must age a little more than s_1 scions before the inhibitor level falls below the lower threshold. However, the flowering behaviour of the scions seems to bear no relationship to the genotype at the E locus. Differences such as those between scions 60 and 53 or 24 and 2 were hardly investigated in Part II but they are thought to arise from a difference in content of polygenes modifying the action of S_2 and sundry other genes with small effect.

Slow grafts.

Data for slow grafts involving s_2 scions are rather limited but it is clear that any differences from vigorous grafts of the same type are small and of a quantitative nature. However, in the case of S_2 scions in graft combinations where vigorous grafts flowered at an early node (e.g., 24/58, 53/60 etc.) the slow grafts flowered at a late node in 23 cases out of 24. That is in contrast to the s_2 scions there is a qualitative difference between the majority of vigorous grafts and the slow grafts. Presumably, such slow growing scions receive very little in the way of metabolites from the stock cotyledons. Their late flowering node suggests that the cotyledons in normal grafts may supply either a positive stimulus or at least a background of substances essential to flowering. On the other hand late flowering in slow grafts could be caused by relatively high concentrations of inhibitor building up as a result of the very slow growth rate. Slow grafts are probably equivalent to cuttings or perhaps even embryos excised at the same state. Five L60 shoots were cut off above the cotyledons at 6 days and grown as cuttings under SD's. One of these cuttings also flowered at a high node similar to that of slow grafts of 60/60. Intact L60 plants and vigorous 60/60 grafts flower at an early node. The evidence from cuttings also suggests that the ES_2 cotyledons of L60 supply a promotor.

Conclusion.

The results for vigorous grafts may all be explained under the inhibitor-only scheme by the proposals (1) that S_2 produces inhibitor in the cotyledons and shoot, (2) that gene E lowers the level of inhibitor in the cotyledons and (3) that gene S_1 increases apical sensitivity to inhibitor. However, the results for slow grafts suggest that a promotor is also involved. If a promotor-inhibitor scheme is required, the balance model seems preferable for reasons given in Chapter 5. All the graft results may be accommodated by switching to the balance model and including in the proposals the assumption that the cotyledons and shoots of all varieties used are equally capable of producing promotor. The behaviour of L60 (s_1ES_2) and 60/60 grafts and cuttings, for example, could be explained under these proposals as follows.

Inhibitor levels in 60 cotyledons are fairly low because E counteracts the presence of S_2 . E may suppress S_2 activity or destroy S_2 -product. With intact 60 plants and vigorous 60/60 grafts abundant supplies of promotor turn the balance in favour of flowering at around node 11. It is possible that the apex is vegetative up to this node because inhibitor is mobilised more rapidly than promotor. The scion continues to flower because although inhibitor production by S_2 in the scion tissue is not counteracted by gene E, the large reserves of promotor in the cotyledons maintain a favourable balance until S_2 activity is inevitably diminished by aging. With this model, the highest levels of inhibitor might be expected at the region where the cotyledonary influence is waning and the aging effect is only starting to gain strength. This region would probably be around nodes 14 - 17 and these proposals will explain the occurrence in many s_1ES_2 plants of vegetative reversion in the 14 - 17 region. The bimodality of L61 (s_1eS_2) may also be explained in the same manner because the minimum frequency region in the distribution is also in the region of node 17. (Gene S_2 shows incomplete penetrance in L61. A distribution of flowering

node may be seen in Table 9, p. 55). With L60 cuttings or 60/60 slow grafts the situation at the apex is largely dependent on the potentialities of the shoot itself. Without the extra supply of promotor from the cotyledons, gene S_2 maintains in the shoot a balance unfavourable to flowering until the inhibitor level falls with aging. These proposals lead to the prediction that removal of one cotyledon in s_1ES_2 plants will increase the frequency of vegetative reversion. It should increase the penetrance in L61.

The promotor-only scheme was originally passed over, as S_2 , E and S_1 were all required to be regulatory genes when it is easier to picture S_2 as a structural gene and because it was also necessary to assume the presence of the structural gene for promotor in all varieties. However, as the last assumption is now required with the balance model and it is also necessary to assume small quantities of inhibitor in s_2 plants, it is almost as simple to use a promotor-only scheme. Suppose that dominant S_2 reduces promotor formation in the cotyledons and shoot, E suppresses the action of S_2 in the cotyledons and S_1 reduces the sensitivity of the apex to promotor. Such proposals will also explain all results. Take for example the graft 24/58. L58 cotyledons (s_1es_2) supply abundant promotor which induces 24 scions (S_1eS_2), which are low in promotor, to flower about node 12. However, young 24 scions contribute no promotor and as the supply from the donor cotyledons falls off the promotor level drops below the threshold and rises above it once again only when the action of gene S_2 is diminished by the aging process.

Thus, there remain alternative proposals for the action of S_2 , E and S_1 and the control of flowering in Pisum. The balance model is perhaps the more complicated alternative but when considered in regard to other published work, it may be the most satisfactory. This question is discussed in Chapter 7. However, it does appear that gene S_2 operates in both the cotyledons and the shoot and gene E in the cotyledons only and that gene S_1 governs some property of the shoot.

Fig. 3. Mean node of first flower $\bar{x} \pm SE$ for the 36 experimental grafts and 6 ungrafted controls. The means are given numerically in the first column of Table 13 and any standard errors not shown and sample sizes may be found in the second and third columns of Table 13.

FIGURE 3.

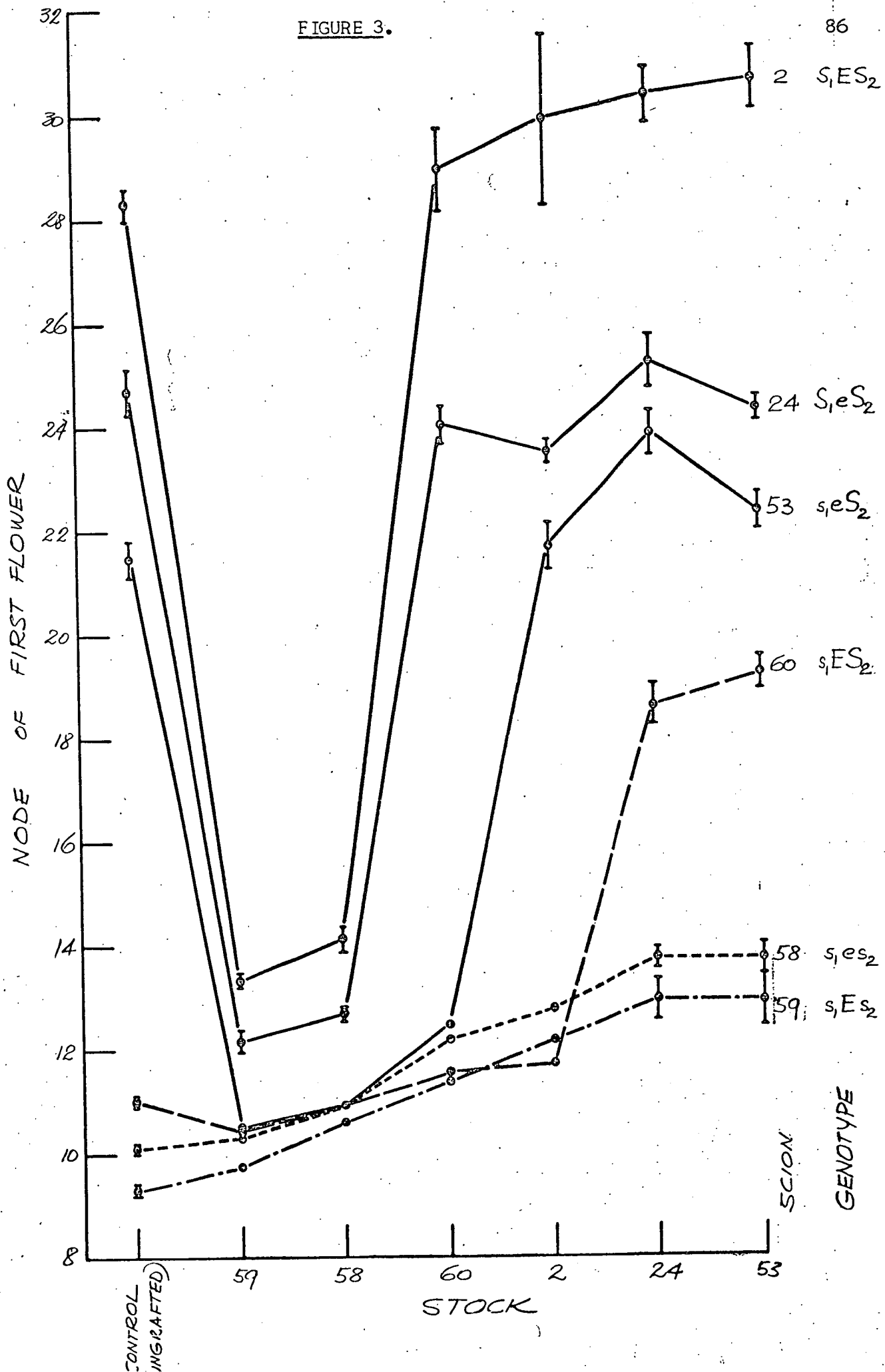


Fig. 4. shows the reproductive state at nodes 0 through 35 for scions 60, 53, 2 and 24. Open column represents the vegetative condition and shaded column the flowering condition. Sample sizes are shown to the left of the columns, e.g., in graft 60/59 12 scions flowered at an early node of which 3 subsequently reverted to vegetative for an interval before commencing permanent flowering. The change points and their standard errors are shown numerically in Table 13.

<u>Genotypes</u>	59 s_1Es_2 ,	58 s_1es_2 ,	60 s_1ES_2 ,
	2 S_1ES_2 ,	24 S_1eS_2 ,	53 s_1eS_2 .

FIGURE 4.

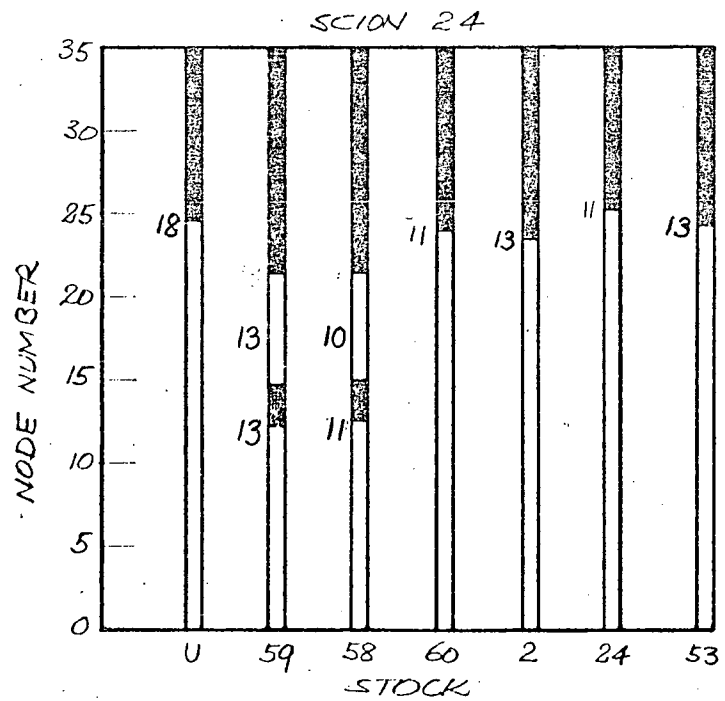
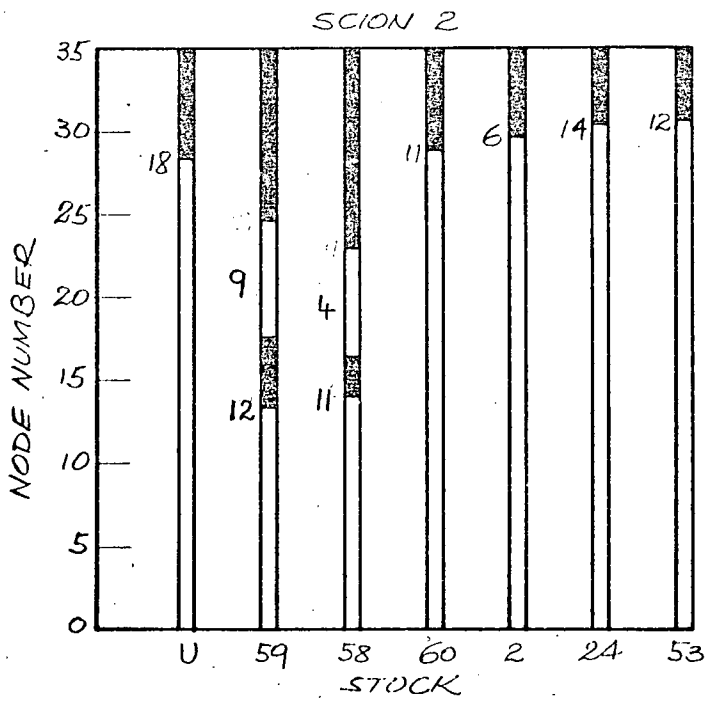
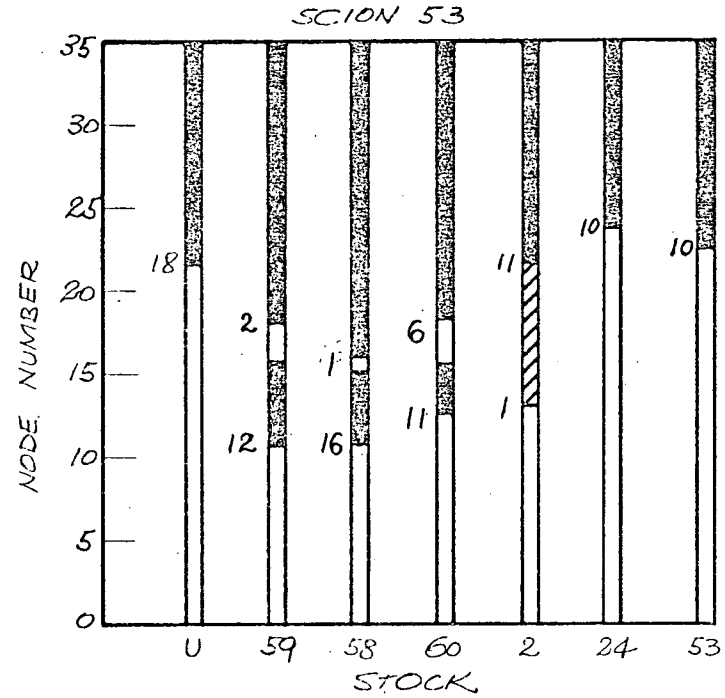
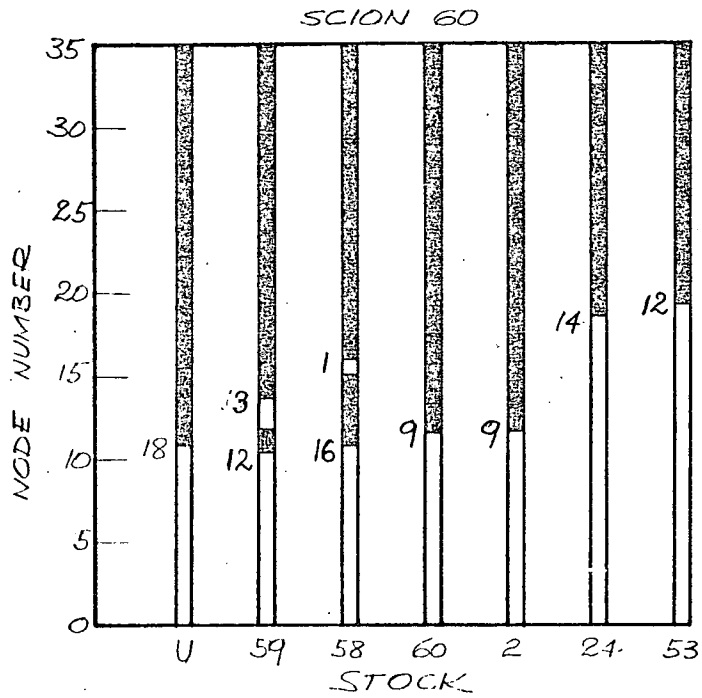


Table 13.

Data on the flowering behaviour of scions from the reciprocal grafting experiment. 59/58 indicates a scion of Line 59 grafted to a Line 58 stock. Genotypes - 59 s_1Es_2 , 58 s_1es_2 , 60 s_1ES_2 , 2 S_1ES_2 , 24 S_1eS_2 and 53 s_1eS_2 . The meaning of the columns may be illustrated by treatment 24/59. There were 18 grafts of which 15 grew vigorously, 1 grew slowly and 2 failed altogether. 13 vigorous grafts flowered at an early node. All 13 subsequently reverted to vegetative and commenced flowering for a second time, this time permanently at a late node. 2 vigorous grafts did not flower at all until a late node and are listed as 'atypical'.

Treatment	Node of 1st flower				Vegetative Reversion								Atypical grafts				Slow grafts				n	Graft failed (n)
	\bar{x}	\pm	SE	n	Revert \bar{x}	\pm	SE	to veg.	Resume \bar{x}	\pm	SE	flowering	n	\bar{x}	\pm	SE	n	\bar{x}	\pm	SE	n	
59	9.33	0.14		12	10.00				11.00				1				0				0	0
59/59	9.75	0.25		8									0				0				0	4
59/58	10.57	0.30		7									0				0				0	5
59/60	11.36	0.32		8	12.00	0.00			13.00	0.00			3				0	11.00	0.00		2	2
59/2	12.17	0.17		12	13.00	0.00			14.00	0.00			2				0				0	6
59/24	12.90	0.38		10	15.00				16.00				1				0	14.00			1	1
59/53	12.88	0.48		8	11.00				13.00				1				0	11.00			1	3
58	10.08	0.08		12									0				0				0	0
58/59	10.33	0.21		6									0				0	11.50	0.50		4	2
58/58	10.90	0.18		10									0				0	11.00	0.00		2	0

Table 13 (Continued)

Treatment	Node of 1st flower				Vegetative Reversion								Atypical grafts				Slow grafts				Graft failed(n)
	\bar{x}	\pm	SE	n	Revert \bar{x}	to veg. \pm	Resume flowering \bar{x}	\pm	SE	n	\bar{x}	\pm	SE	n	\bar{x}	\pm	SE	n			
58/60	12.17	0.17		6						0				0				0	6		
58/2	12.75	0.25		12	12.00		13.00			1				0	12.50	0.50		2	4		
58/24	13.71	0.18		7						0				0	13.00	1.00		2	3		
58/53	13.70	0.30		10						0				0	14.00			1	1		
60	11.00	0.11		18						0				0				0	0		
60/59	10.42	0.21		12	11.67	0.33	13.67	0.33		3				0				0	6		
60/58	10.94	0.14		16	15.00		16.00			1				0				0	2		
60/60	11.56	0.29		9						0				0	18.00	1.00		2	7		
60/2	11.67	0.17		9						0				0	18.64	0.59		11	4		
60/24	18.57	0.36		14						0				0	18.50	0.50		2	2		
60/53	19.17	0.30		12						0				0	18.00	0.71		4	2		
2	28.28	0.60		18						0				0				0	0		
2/59	13.33	0.14		12	17.56	0.71	24.78	0.60		9				0	25.00			1	5		
2/58	14.09	0.25		11	16.25	0.48	23.00	1.73		4	26.00	0.00		2	26.50	1.50		2	3		
2/60	28.91	0.78		11						0				0	27.50	1.50		2	5		
2/2	29.83	1.62		6						0				0	28.40	0.81		5	7		
2/24	30.29	0.53		14						0				0	29.50	0.50		2	2		
2/53	30.58	0.57		12						0				0				0	6		

Table 13 (Continued)

Treatment	Node of 1st flower				Vegetative Reversion								Atypical grafts				Slow grafts				Graft failed(n)
	\bar{x}	\pm	SE	n	Revert to veg.	Resume flowering							\bar{x}	\pm	SE	n	\bar{x}	\pm	SE	n	
24	24.67	0.46		18								0				0				0	0
24/59	12.15	0.22		13	14.69	0.35	21.31	0.31	13				21.00	0.00	2		21.00			1	2
24/58	12.64	0.15		11	15.00	0.58	21.40	0.65	10				21.60	0.81	5		14.00			1	1
24/60	24.00	0.36		11					0						0		23.20	0.37		5	2
24/2	23.46	0.22		13					0						0		24.00	0.71		4	1
24/24	25.18	0.50		11					0						0		23.50	0.87		4	3
24/53	24.23	0.28		13					0						0		24.00	1.00		2	3
53	21.44	0.35		18					0						0					0	0
53/59	10.50	0.15		12	15.50	0.50	18.00	0.00	2						0		19.00			1	4
53/58	10.88	0.13		16	15.00		16.00		1						0					0	2
53/60	12.45	0.28		11	15.33	0.42	18.17	0.79	6				19.00		1		19.60	0.40		5	1
53/2	13.00	0.00		1					0				21.64	0.45	11		21.50	1.50		2	4
53/24	23.80	0.42		10					0						0		21.40	0.87		5	3
53/53	22.30	0.34		10					0						0		20.50	1.50		2	6

PART IV.

GENERAL DISCUSSION.

CHAPTER 7.

Von Denffer (1950) raised the general question of "Bluhhormon oder Bluhhemmung?". Since that time several workers have claimed evidence for the involvement of a flower inhibitor in the control of flowering in Pisum. However, this interpretation has been challenged by others who favour a scheme involving flower promotor only.

Papers suggesting a flower inhibitor in peas.

Existence of a flower inhibitor (colysanthin) in the cotyledons of late pea varieties was proposed by Barber and Paton (1952) and Paton and Barber (1955) from the results of experiments involving intervarietal grafting and cotyledon removal. Amos and Crowden (1969) obtained similar data using the same varieties

Footnote. The garden pea cultivars Massey (early dwarf) and Greenfeast (late dwarf) have been widely used in Australia for research on flowering. Line 24 (S_1eS_2) was derived by several generations of single plant selection from cultivar Greenfeast and Line 59 (s_1Es_2) was derived in the same way from cultivar Massey. However it cannot be assumed that the commercial varieties have these genotypes. The batch of commercial Massey used originally in this crossing programme was certainly heterogeneous for alleles E and e. In addition it is understood that wilt resistance was bred into 'Greenfeast' around 1960. Also, Rowlands (1964) suspected heterogeneity in several commercial varieties used in his crosses. This raises doubts as to the consistency and purity of commercial cultivars and makes difficult the comparison of results from workers using apparently identical material. The different genotypes within cultivar Massey are phenotypically indistinguishable but they may not react the same way to all experimental treatments. It would seem desirable that physiological experiments are carried out with genetically known pure varieties.

Massey (M) and Greenfeast (G). The results given in the above three papers differ from the graft results reported here and by Kohler (1965) in two respects; the qualitative type of response as shown by say 24/58 was not observed and there is no mention of vegetative reversion. The difference in results is probably not due to genotypic differences as graft G/M is believed equivalent to 24/58 or 24/59 (See footnote, previous page). It possibly arises from differences in time of grafting or experimental conditions. Paton and Amos grafted at 6 - 8 days as opposed to 4 days in the present case. However grafts of 24/59 made at 6 days still showed a qualitative response. Again the percentage of 24/59 grafts which show a qualitative response varies from experiment to experiment due presumably to some uncontrolled variation in experimental conditions but in seven different experiments under SD's the percentage was never less than 50 %.

Vegetative reversion was observed by Paton (1956) in Greenfeast cuttings which were grown for two weeks in LD's and then transferred to SD's. He suggested that there is some production of the flower inhibitor in the leaves of late varieties under SD conditions. Vegetative reversion was also observed by Barber (1959) in Greenfeast seedlings which were raised for some time under SD's, transferred for a week into continuous light and then returned to non-inductive SD conditions. These results agree with the action of gene S_2 proposed here.

Sprent and Barber (1958) concluded from a leaching experiment with Greenfeast cuttings that the flower inhibitor passes from the cotyledons of late varieties into the plumule over the first fortnight of growth.

Barber (1959) investigated the genetics of flowering and interaction of genotype, photoperiod and vernalisation. He concluded that his late varieties differed from the early varieties in possessing a dominant gene Sn which
 "(a) delays flowering to a higher node; induces the competence to respond to
 (b) photoperiod and (c) vernalisation; (d) delays the appearance of ^{the} first leaf

with four leaflets and (e) reduces growth in length of stem". Gene S_2 may be responsible for all the pleiotropic effects which Barber attributes to Sn but some of these properties have not been checked here. It is clear that S_2 delays flowering node and flowering time, it suppresses floral development under SD's and it delays senescence. S_2 also confers the ability to respond to photoperiod but whether S_2 is the only gene which gives this ability is not known. The response of genotype S_1es_2 to photoperiod and vernalisation has not been tested but if S_1 controls apical sensitivity as suggested this genotype may perhaps respond to vernalisation but not to photoperiod. Again whether the response to photoperiod is actually mediated via the S_2 gene is not entirely certain. Haupt (1969) has suggested that early varieties initiate so soon after germination that they have no opportunity to respond to photoperiod. The absence of photoperiod response in Massey embryos which are exposed to light from the first day (Johnston and Crowden 1967) or in Massey seedlings which are delayed to node 11 or 12 by gibberellic acid (Barber et al 1958) suggests that the ability, and not just the opportunity, is conferred by gene S_2 .

Paton (1956), Sprent (1958) and Barber (1959) have each proposed schemes in which florigen (\emptyset) and colysanthin (K) may be converted one to the other. The latter two workers proposed that the Sn gene converts \emptyset into K. It has been argued here that Sn and S_2 are the same gene. The results in Chapter 6 indicate that gene S_2 operates in shoot tissue as well as in the cotyledons. Evidence, both already given and to come, indicates that the cotyledons of early varieties such as Line 58 (s_1es_2) are a source of flower promotor. If S_2 converts \emptyset to K, Line 53 scions (s_1eS_2) grafted to Line 58 stocks should flower late or if induced to flower early they would be expected to quickly revert to vegetative. In fact the great majority of 53/58 grafts flowered permanently from an early node (See Fig. 4, p. 88 and Table 13, p. 89-91).

Sprent (1966) has shown that cotyledon removal in the late pea cultivar Greenfeast within 12 days from the start of germination results in a reduction

of the flowering node. She suggests that a flower inhibitor in the cotyledons is quantitatively transported to the apex during the first 2 weeks of growth. She has also shown that defoliation in peas may cause a substantial change in stem-length, whilst causing very little or no change in flowering node. Suppose, as Sprent suggests, that the contribution of the leaf is a function of the photoperiod or ratio of red to far-red light. The absolute level of leaf product(s) might still vary with the leaf area. However, with the balance model, the absolute quantities of ϕ and K may vary with the leaf area whilst the proportion of $\phi : K$ may be independent of the leaf area and dependent only on the photoperiod.

Johnston and Crowden (1967) whilst supporting the idea of a flower ^{the} inhibitor in cotyledons of late varieties concluded that "colysanthin and photoperiod have an independent effect". They have grown excised embryos of Massey and Greenfeast under different photoperiods. Their data show that in the late variety Greenfeast (probably S_1eS_2) cotyledon removal lowers the flowering node but the response to photoperiod is similar for excised embryos and intact control plants even when the embryos are excised 8 hours after the start of imbibition. Their conclusion that colysanthin and photoperiod have an independent effect follows if the cotyledons are assumed to be the sole source of inhibitor. In fact their data support the present suggestion that the S_2 -substance (inhibitor) is formed in the shoot as well as in the cotyledons, the level of inhibitor being influenced by the photoperiod.

They also found that Greenfeast embryos excised at 8 hours flowered at node 22 under SD's. With the inhibitor-only model, these embryos would be expected to behave like the 24/58 grafts by flowering at 12 and later reverting to vegetative till around node 20 or 22. However with the balance model, removal of the cotyledons not only lowers the level of S_2 -substance, it also lowers the level of the flower promotor. The $\phi : K$ ratio therefore remains unfavourable to flowering. The large cotyledons of peas presumably exert a

considerable influence over the early life of the plant and the growth of embryos is very different to that of intact plants.

Embryos of the early variety Massey excised at 8 hours flowered at the same node as intact plants but embryos excised at 1, 2 or 4 days flowered at a significantly higher node (an increase 1 - 2 nodes). Johnston and Crowden suggest that Massey cotyledons may contain a flower inhibitory substance which is rapidly mobilised after germination commences and which is subsequently deactivated or converted to a promotive substance. These results may be accommodated by the balance model as follows. Massey cotyledons are a source of both promotor and a small quantity of inhibitor but the latter is more rapidly mobilised. Embryos excised at 8 hours possibly contain less promotor and inhibitor than intact plants but they flower at the same node because it is the relative balance and not the absolute quantities which determine initiation. Haupt (1952, 1957) and Moore (1964) have also found that cotyledon removal may raise the flowering node of early varieties. However, the behaviour of *Kleine Rheinlanderin* (KR), the early variety used by Haupt, seems to be substantially different from that of Massey and the behaviour of KR is discussed more fully later on.

Paton (1969) has published results in which unvernalsed Greenfeast stocks delayed Massey scions by 2.9 nodes in SD's ($P < 0.001$) and 1.1 nodes in LD's ($P < 0.05$). Vernalised stocks had no significant delaying effect. These results support the concept of a transmissible flower inhibitor in the cotyledons of Greenfeast which is either destroyed by vernalisation or, as Paton suggests, whose synthesis is repressed by vernalisation. The data also indicate that this inhibitor may confer sensitivity to photoperiod on the Massey scions as would be expected on the Barber hypothesis or the model proposed here but Paton reports that he has been unable to consistently obtain this effect. (Is the lack of repeatability due perhaps to inconsistency in the genotype of commercial varieties?). Secondly, Paton has shown that unvernalsed Greenfeast plants have

a three day delay between induction and initiation, the delay being non-existent in vernalised plants. He concluded that vernalisation of Greenfeast leads to the absence of a floral inhibitor which is present in unvernalsed plants where it delays rapid evocation at the apex. However, as Amos and Crowden (1969) have shown that embryos of Greenfeast excised at 8 hours can still show a substantial response to vernalisation, the vernalisation effect is not solely mediated via inhibitor, at least not inhibitor from the cotyledons.

These workers claim that "vernalisation appears to have two separate effects both of which promote flower initiation at a lower node. The smaller effect is manifest on the cotyledonary inhibitor system, and probably results from a reduction of the effective level of colysanthin. The major effect does not appear to involve colysanthin, but is manifest on the young embryo and is effective before photoperiodic induction is completed". Amos and Crowden have not considered the possibility that the embryo tissue may also act as a source of colysanthin. If, as proposed here, gene S_2 operates in both the cotyledons and the shoot then it is possible to explain their data in terms of a single effect, namely a general reduction in the activity of gene S_2 brought about by vernalisation. However, Paton's transfer experiment indicates that vernalisation conditions the apex in some way. It was suggested here that gene S_1 governs apical sensitivity to inhibitor or promotor. Greenfeasts' genotype is probably S_1eS_2 . These remarks lead to an interesting speculation. Are there two vernalisation effects; one achieved by a reduction in S_2 activity and the second achieved by suppression of gene S_1 ? This speculation cannot be tested until a pure line of genotype S_1es_2 is developed.

Papers favouring a promotor-only system in peas.

The argument in favour of a promotor-only scheme has been developed by Haupt (1952, 1954, 1955, 1957, 1969) and Kohler (1965). Their proposals, which are summarised in Haupt (1969) are based on extensive experiments involving

cotyledon removal, embryo culture, cuttings and grafts of various types. They maintain that it is not necessary to include an inhibitor in a model to explain the control of flowering in peas. This is true in the case of the present results and a model of gene action is proposed on page 84 which involves only promotor. However, a scheme employing a single flowering hormone runs into difficulty accommodating certain results. Some of these difficulties are resolved by assuming a promotor-inhibitor balance model and I propose to examine certain points raised by Haupt in relation to Scheme 3.2 and the genetic data presented herein.

Haupt and Kohler propose that there are two possible ways in which a growing tip may initiate flowers, either autonomously or after induction. Early varieties and scions of late varieties grafted to early donors are induced to flower by a florigenic substance from the cotyledons. Late varieties and early varieties deprived of their cotyledons are determined autonomously as the plant ages. Their theory has in common with the present theory the idea of a promotory substance in the cotyledons of early varieties. However, in the present theory late cotyledons are considered to have the same promotory substance(s), flowering being suppressed by a high level of inhibitor which is at a low level in early varieties. Again the idea of autonomous determination in lates has much in common with the idea of flowering in late varieties resulting from an inevitable fall in inhibitor, even under SD's as the plant ages. The main differences are:- (1) the use of both promotor and inhibitor and the balance model (2) the assigning of a central role to an inhibitor and (3) the assumption of a single cause of flower initiation. Graft results in which a late scion on an early stock flowers at a low node, reverts to the vegetative state and finally flowers for a second time at a much higher node have led Kohler to suggest two types of initiation. With the balance model the first and second flowering are determined by the same underlying cause, namely, a favourable $\phi : K$ balance. With a graft of a late scion to early stock the

balance first turns in favour of flowering largely because the early cotyledons push promotor into the system, the balance then becomes unfavourable as the S_2 -scion tissue pushes inhibitor into the system and finally the balance once more favours flowering as the aging process inevitably leads to the destruction of S_2 -substance or the switching-off of the S_2 gene.

Some results on page 443 of Kohler's paper are particularly interesting in regard to the argument for a promotor. Scions of the early variety KR when grafted to stocks of the late variety Alderman (AL) are delayed to a higher node and show a response to photoperiod. Embryos of KR show a very similar behaviour, flowering as late as node 17 under a 4 hour photoperiod. These results certainly seem to favour the view that the cotyledons of the early variety KR contain a promotor which is lacking in the cotyledons of the late variety AL. Embryos of the early variety Massey do not respond to photoperiod and do not flower later than node 11 (Johnston and Crowden 1967). However, as shown in Chapter 6 (or Paton 1969) Massey type scions (Line 58 and Line 59) are delayed up to node 14 by grafting to cotyledons of late varieties (e.g. Line 24 and Line 53). The results with Massey favour the idea of an inhibitor in the cotyledons of late varieties which is absent in the cotyledons of early variety Massey. Obviously Massey and KR give rather different results and the explanation may lie in the genotype. Scions of Line 60 (s_1ES_2) flower at node 10 or 11 on their own cotyledons but at node 18 under SD's when grafted to a stock of late varieties Line 24 or Line 53 (Chapter 6). In a small pilot experiment Line 60 shoots similar in age to those used in the grafts were planted as cuttings. Some of these also flowered at node 18 under SD's. These results with Line 60 again appear to favour the idea of a promotor in 60 cotyledons which is absent in 24 or 53 cotyledons. (It is tempting to suggest that gene E gives promotor but this suggestion is not supported by the fact that genotypes s_1es_2 and s_1ES_2 have closely similar flowering nodes). These apparently contradictory results may be explained by the balance model after taking account of the genotypes.

It is proposed that Line 58 ($s_1 es_2$) cotyledons are predominantly a source of promotor but they also possess small quantities of inhibitor. Line 60 ($s_1 ES_2$) cotyledons are an equal source of promotor and a little richer in inhibitor on account of their genotype ES_2 . The two scions also have a similar ability to produce promotor but differ in the ability to produce inhibitor which is lacking in Line 58 (gene S_2 absent) and present in Line 60 (gene S_2 present). With Line 58 plants, whether intact, cuttings or embryos, the $\phi : K$ balance turns in favour of flowering by node 11. With Line 60 cuttings the balance is no longer dominated by promotor from the cotyledons and is dependent on the potentialities of the scion. The presence of gene S_2 leads to a balance dominated by inhibitor until about node 18 under SD's. Cuttings were not grown under LD's. Embryos of Line 60 have also not been grown but on this theory one might predict them to flower as late as node 18 under SD's and also to show a response to photoperiod. Line 60 scions grafted to Line 53 stocks are late because the balance emerging from both the cotyledons and the scion tissue favours the vegetative state. Perhaps the behaviour of KR may be explained along these lines although this is not to imply that KR and Line 60 have the same genotype.

My results differ from those of Kohler in the following respect. In grafts of the type AL/KR he found that reversibly induced scions flowered for the second time at the same node as the controls and scions which were not induced to flower early, initiated their first flower. That is 'autonomous' initiation in AL scions was not affected by grafting. In the present experiment reversibly induced scions flowered for the second time at the same node as scions which were not induced to flower early, initiated their first flower but this was some 3 - 6 nodes lower than the controls. (See Table 13, p. 89, 91 and Fig. 4, p. 88). A qualitative type of response was not observed in the experiments of Paton and Barber (1955), Paton (1956) and Amos and Crowden (1969) but late scions grafted to early stocks flowered a few nodes earlier than the controls

which finding agrees with the present results. The reduction in flowering node may occur because a lower total quantity of inhibitor has entered the system. However, if the flower-regulating-substances are short lived then the cotyledons would probably have little influence on flowering behaviour above node 18.

The relatively strong influence of the cotyledons over the first fortnight of growth is shown by the parallel behaviour of scions 59 and 58 grafted to stocks 59, 58, 60, 2, 24 and 53. (Fig. 4). It is clear that the flowering node of s_2 scions is strongly influenced by the genotype of the stock. The flowering behaviour of S_2 scions is also strongly influenced by the stock in some cases (compare 2/58 and 2/60), but where S_2 scions flower above node 18 the effect of the stock is relatively minor (compare scion 24 on stocks 60, 2, 24 and 53) in comparison with the influence exerted by the genotype of the scion itself (compare scions 60, 53, 24 and 2 on stock 24). It seems that the flowering node above node 18 is largely determined by the genetic potentialities of the scion. If the word largely were omitted from the previous statement, it would agree with Kohler's results. However the majority of results suggest that some stocks significantly influence 'autonomous' initiation in late scions.

With the varieties used by Kohler an 'induced' late variety, e.g., AL/KR, always flowers later than an 'induced' early variety, e.g., KR/KR. However, this statement cannot be generalised as for example 53/58 flowers at the same node as 58/58 (Fig. 3, p. 86). The difference is presumably a question of genotype. Scions 53 and 58 carry recessive s_1 and are thought to have the same low sensitivity to inhibitor. Consequently grafts 53/58 and 58/58 flower at the same node. Scions 2 and 24 carry dominant S_1 which increases apical sensitivity to inhibitor. Consequently grafts 2/58 and 24/58 flower 2 - 4 nodes later than 58/58. (With the balance model low sensitivity to ϕ is equivalent high sensitivity to K). The small difference between s_1 scions 59 and 58 or S_2 scions 2 and 24 is presumably caused by differences in the background of minor

genes. Possibly AL carries S_1 and KR gene s_1 .

Haupt (1958) has found that late receptors, with or without their cotyledons, are made earlier by grafting to early donors. Promotion is stronger in receptors without cotyledons. Haupt points out that no effect at all would be expected for receptors with cotyledons if only colysanthin were to be considered and the results therefore point towards the donor as a source of promotor although he also admits the possibility that the receptor is contributing a colysanthin effect. Any conflict in these interpretations is resolved by the balance model which accommodates the results as follows. Both donor and receptor cotyledons supply promotor but inhibitor is supplied for the most part by the receptor only. The relative promotor level is therefore raised and balance turns more readily in favour of flowering. In respect of these experiments Paton (1956) found that under SD's Greenfeast receptors with cotyledons actually flowered slightly later when grafted to Massey donors with cotyledons than Massey stocks without cotyledons. However, the LD results were again in agreement with those of Haupt.

Examination of the results in Chapter 6 and a number of the papers just discussed shows that scions of late varieties grafted to early stocks flower at a very much lower node than cuttings or embryos of the same late variety or slow grafts of the same type. As remarked by Haupt (1969) such data point towards the contribution of a positive floral stimulus by the cotyledons of early stocks.

Some examples from other plants.

The discussion to this point has concerned only peas. The general question of a flower inhibitor was raised by von Denffer (1950) and Barber (1959) has discussed the subject from a genetic angle. Arguments against specific floral inhibitors produced under non-inductive conditions are summarised by Zeevaart (1963). However, evidence for the existence of transmissible flower inhibitors is mounting and a few relevant papers are mentioned below.

Guttridge (1959a and b) and Thompson and Guttridge (1959) have strong evidence for a transmissible flower-inhibiting, vegetative-growth-promoting substance in strawberries. This substance seems to have much in common with the substance produced by the S_2 gene. Further in both peas (Barber et al 1958) and strawberries gibberellic acid has several properties in common with the inhibitor although in both cases the authors decided that the inhibitor was not itself gibberellic acid. The argument that the gibberellins are involved, at least marginally, with the flowering reactions in peas is supported by the fact that the Cy_1 gene had a significant effect on flowering behaviour in Crosses 2 and 126. Brian (1957) and Sprent (1958) have already shown that the Cy factors are involved with gibberellin metabolism.

Resende (1949, 1959), working with succulents, has proposed that the floral state differs from the vegetative state only quantitatively and that these states are not characterised by specific hormones, only a different hormone balance. Schwabe (1956, 1959) has proposed that in Kalanchoe a substance is formed under LD's which inhibits florigen formation. Raghavan and Jacobs (1961) have results which suggest that flowering in Perilla may be regulated by a balance between promotor and inhibitor. Evans (1960) and Evans and Wardlaw (1964) propose that the leaves of Lolium in SD's produce a transmissible flower inhibitor which interacts at the apex with a transmissible stimulus to inflorescence initiation formed in leaves exposed to LD's. Kijosawa and Kijosawa (1962) propose from grafting experiments that flowering in soybeans is determined by a promotor-inhibitor balance.

Concluding discussion.

In conclusion the results in peas seem in some cases to favour a promotor and in other cases an inhibitor. The promotor-inhibitor balance model would seem to accommodate the results more easily than a scheme involving either promotor or inhibitor only. The claim for an interaction between promotor and

inhibitor is certainly not isolated to peas as shown by the references to certain succulents, Perilla, Lolium and soybean. That is not to imply that exactly the same substances are involved in all flowering plants but perhaps the same classes or families of substances are concerned. Again the idea of a balance would seem to be in keeping with recent views on the broader field of hormonal regulation in higher plants. The theory that growth and development are regulated by interactions between promotive and inhibitory hormones is discussed by Galston and Davies (1969). (The words balance and interaction are used somewhat as alternatives. I have assumed that the relative proportions are important in a balance. All balances involve an interaction but all interactions do not involve a balance).

The fact that there seems to be no genetic variation in promotor production in peas may be considered a weakness in the present scheme. Adaptive genetic control can be achieved by selection of genes governing different aspects of the process. Thus one species may regulate flowering via the promotory component; another species may show variation in the gene systems controlling the inhibitory component. However, one of the possible actions of S_1 is to lower the level of promotor. Again not all genetic variation is covered in this report and more genes are certain to be uncovered some of which may be concerned with a promotor.

A genetic study is only as good as the screening methods used to recognise the genetic variants. The present study made use of a special environment (short days) and two variables (time to open flower and node of first initiated flower irrespective of development). The varieties were not scanned for response to vernalisation and this treatment was not used to distinguish variants. The use of more physiological information will probably lead to the recognition of further genetic variants perhaps even amongst the plants genotyped in this study. On the other hand, the genetic information gained

here opens up the possibility of further physiological and biochemical studies. For example by using known genotypes and electrophoretic techniques it may be possible to associate protein differences with particular genetic variants. There may be qualitative differences between genotypes which will allow the recognition of at least some enzymes involved in flowering control. But if the balance theory is correct, within one genotype there may be only quantitative differences between vegetative and flowering plants. Some results of Marushige and Marushige (1962) and Nitsan (1962) are of interest in this respect. They found that extracts from flowering and vegetative plumules of Pharbitis showed quantitative but not qualitative differences in protein electrophoresis patterns which could imply that only slight changes in the balance of enzymes already operating in the bud determine the difference between the vegetative and flowering states and that new proteins are not essential.

The BALANCE MODEL of flowering control (Scheme 3.2).

The flowering and vegetative states are determined by the balance between promotor (\emptyset) and inhibitor (K).

Flower initiation occurs when

$\emptyset > \text{threshold } T > 0$, $K > \text{threshold } T' > 0$ and $\emptyset : K > \text{some constant } R$.

For further discussion of the model and variations including the concept that minimal quantities of both \emptyset and K are necessary for bud morphogenesis see p. 64 .

SUMMARY.

(1) The genetics of flowering in nine pea varieties was investigated by extensive inter-crossing. Some crosses were followed to the sixth generation. A theory of gene action was built up from the crossing data and tested physiologically by reciprocal grafting between known genotypes. This theory is then discussed in relation to other published work.

(2) Under short days the varieties used in the crossing programme may be grouped into three distinct classes;

- (i) ED (early developing) plants initiate and develop flowers at a low node
- (ii) L (late) plants initiate and develop flowers at a high node and
- (iii) EI (early initiating) plants initiate flower buds at a low node but as the lower flower buds fail to develop, the time of open flower is similar to that of L plants.

These classes are defined in relation to three type-varieties.

(3) Class-differences are controlled by three dominant major-genes, S_1 , E and S_2 , which interact as follows. The triple recessive is an ED-type. Addition of S_2 creates an L-type. E is epistatic to S_2 in terms of flowering node and genotype s_1ES_2 is an EI-type. S_1 is epistatic to E and S_1ES_2 is again L-type. S_1eS_2 is also L-type. S_1 and E have little or no effect by themselves and genotypes S_1es_2 , s_1Es_2 and S_1Es_2 are essentially ED.

(4) Genes S_1 and E segregate from their recessive alleles in a Mendelian manner but segregation of the S_2/s_2 pair is significantly disturbed, with an average of only 20 % recessive plants in F_2 . The disturbance is not caused by differential viability or misclassification. S_1 is linked to the A gene for anthocyanin with an RCV of 9 % and gene E is linked to the P gene for pod membrane with an RCV of 22 %. The linkage of S_2 was not detected as it recombines freely with the six markers tested. (A, I, Cy₁, V, P and R).

(5) There are several examples of maternal influence. In particular, the genotype $S_1-ees_2s_2$ seems to flower a few nodes earlier when derived from mothers carrying gene S_2 than it does if derived from mothers lacking S_2 .

(6) Gene S_2 has several pleiotropic effects. It delays flower initiation to a higher node in s_1eS_2 plants, it suppresses development of the lower flower buds in s_1ES_2 plants under short days, it confers the ability to respond to photoperiod and it promotes continued growth of the plant after flowering and seed set have commenced. The ability to respond to photoperiod is seen in terms of both flowering node and flowering time in L plants but is seen only in respect of flowering time in EI plants. As a consequence of these primary effects gene S_2 greatly increases the total number of nodes, the total height (average increase 540 % in one cross) and the yield (average increase 600 % in one cross). In general gene S_2 opposes flowering and senescence.

(7) The penetrance of gene S_2 in the combination $s_1s_1eeS_2^-$ may be modified in terms of flowering node by both environmental factors and polygenes. (Genotype $s_1s_1eeS_2^-$ classifies EI instead of L). Penetrance is not affected by dosage of S_2 .

(8) Within-class variation derives from (a) combination and dosage effects for the major genes S_1 , E and S_2 , (b) pleiotropy of other major genes not primarily concerned with flowering such as the length gene Cy_1 and (c) various quantitative systems. One quantitative system operates in the complete absence of dominant genes S_1 , E and S_2 . Other quantitative systems are suspected of modifying the action of the major genes.

(9) Vegetative reversion in EI plants was found to be over twice as frequent in cryptodwarf and slender segregates as in dwarf segregates providing a further example of the influence of major internode-length genes on flowering.

(10) Reciprocal grafts between genotypes s_1es_2 , s_1Es_2 , s_1eS_2 , s_1ES_2 , S_1eS_2 and S_1ES_2 were made 4 days from imbibition and grown under an 8 hour photoperiod. Scions either flowered earlier than node 15 (classed early) or later than node 17 (classed late). Scions tended either to grow well (vigorous grafts) or in a weak and retarded manner (slow grafts). Vigorous and slow grafts were analysed separately.

(11) Graft-responses in vigorous grafts could be divided into two types - quantitative or qualitative. Quantitative (within-class) responses ranged from small (about 1 node) as in the case of self-grafts to large (about 3 nodes) illustrated by the delay to s_1-s_2 scions caused by $-eS_2$ stocks. Qualitative (between-class) responses only occurred with S_2 scions. For example, s_1ES_2 scions are early on their own cotyledons but late when grafted to $-eS_2$ stocks (average delay 8 nodes). In contrast S_1ES_2 scions which are late on their own cotyledons flowered early when grafted to s_1-s_2 stocks (average promotion 16-17 nodes). S_2 scions induced to flower at a low node by grafting, frequently reverted to the vegetative state after flowering for only one to several nodes. These scions entered a second, and this time stable, flowering phase some 3 - 6 nodes earlier than self-grafted controls entered their first (and stable) flowering phase. Bursts of vegetative reversion were more frequent and prolonged in S_2 scions carrying dominant S_1 .

(12) Slow grafts of S_2 scions were almost always late even where comparable vigorous grafts were early. Slow scions were thought to receive little support from the stock cotyledons and it is suggested that the stock cotyledons in vigorous grafts supplied a flower promotor.

(13) The flowering behaviour of scions was found to be independent of the genotype at the E locus but dependent on the genotype at the S_1 and S_2 loci. The effect of the stock was independent of gene pair S_1/s_1 but dependent on the genotype at the S_2 locus and also at the E locus when dominant S_2 was present. Therefore gene S_2 is active in both the shoot and the cotyledons whilst gene E is active in the cotyledons only and the S_1/s_1 pair apparently govern some property of the shoot. Activity of gene S_2 in the shoot is also indicated by vegetative reversion in S_2 scions and intact plants of genotypes $s_1s_1E-S_2-$ and $s_1s_1eeS_2-$ (impenetrant types).

(14) A scheme of flowering control is proposed in which flowering occurs when the balance between a flower promotor ϕ and a flower inhibitor K is greater than some critical ratio. It is suggested under this scheme that dominant S_2 produces in the cotyledons and shoot, a substance which inhibits flower initiation and development and which opposes senescence. The level of this substance is reduced by long days and also by the inevitable aging of the plant. Small quantities of inhibitor are assumed in recessive s_2 plants. Gene E is thought to lower the level of inhibitor in the cotyledons. Gene S_1 gives, in effect, high apical sensitivity to inhibitor and gene s_1 the reverse effect. The structural gene for promotor is assumed present in all varieties under consideration. It is not known whether changes in inhibitor level are achieved by repression of gene activity or destruction of gene product. The inhibitor level in the cotyledons is reduced by vernalisation (Evidence from Paton 1969). Inhibitor is thought to be mobilised in the cotyledons more quickly than promotor (Evidence from Johnston and Crowden 1967).

(15) Although the balance model is favoured to explain the present results and other published data, an alternative model is proposed under a promotor-only scheme. If flowering follows when promotor is present above a certain threshold level, then dominant S_2 may reduce promotor formation in the cotyledons and shoot, gene E may suppress the action of S_2 in the cotyledons and S_1 may reduce the sensitivity of the apex to promotor.

(16) It is suggested that the historic symbols Lf and Sn be redefined to take on respectively the meaning which here attaches to S_1 and S_2 . From the linkage between S_1 and A it seems likely that S_1 is identical to the gene originally called Lf. There is no evidence on the relationship of S_2 and the original Sn but replacement of symbol S_2 by Sn seems appropriate on most grounds.

REFERENCES.

- Amos, J.J. and Crowden, R.K. (1969) Effects of vernalisation, photoperiod and the cotyledon on flower initiation in Greenfeast peas. Aust. J. biol. Sci. 22 : 1091-103.
- Barber, H.N. and Paton, D.M. (1952) A gene-controlled flowering inhibitor in Pisum. Nature, Lond. 169 : 592-4.
- Barber, H.N., Jackson, W.D., Murfet, I.C., and Sprent, J.I. (1958) Gibberellic acid and the physiological genetics of flowering in peas. Nature, Lond. 182 : 1321-1322.
- Barber, H.N. (1959) Physiological genetics of Pisum II. The genetics of photoperiodism and vernalisation. Heredity. 13 : 33-60.
- Brian, P.W. (1957) The effects of some microbial products on plant growth. Symp. Soc. Exp. Biol. 11 : 166-82 ed. H.K.Porter.
- Clay, S. (1935) Notes on the inheritance of quantitative characters in a cross between two varieties of garden pea (P.sativum) Jour. Pomol. and Hort. Sci. 13 : 149-189.
- Denffer, D. von. (1950) Blühhormon oder Blühhemmung? Neue Gesichtspunkte zur Physiologie der Blütenbildung. Naturwiss. 37 : 296-301 and 317 - 321.
- Evans, L.T. (1960) Inflorescence initiation in Lolium temulentum L. II Evidence for inhibitory and promotive photoperiodic processes involving transmissible products. Aust. J. biol. Sci. 13 : 429-40.
- Evans, L.T. and Wardlaw, I.F. (1964) Inflorescence initiation in Lolium temulentum L. IV. Translocation of the floral stimulus in relation to that of assimilates. Aust. J. biol. Sci., 17 : 1-9.
- Galston, A.W. and Davies, P.J. (1969) Hormonal regulation in higher plants. Science 163 : 1288-1297.

- Gottschalk, W. (1960) Über züchterisch verwendbare strahleninduzierte Mutanten von Pisum sativum. Der Züchter 30 : 33-42.
- Guttridge, C.G. (1959a) Evidence for a flower inhibitor and vegetative growth promotor in the strawberry. Ann. Bot. (NS) 23 : 351-60.
- (1959b) Further evidence for a growth-promoting and flower-inhibiting hormone in strawberry. Ann. Bot. (NS) 23 : 612-21.
- Hänsel, H. (1954) Versuche zur Vererbung der Nodienzahl-Blühzeit-Relation im langen Tag bei Erbsensorten. (Pisum sativum x Pisum sativum ssp. arvense). Der Züchter 24 : 97-115.
- Haupt, W. (1952) Untersuchungen über den Determinationsvorgang der Blütenbildung bei Pisum sativum. Z. Bot. 40 : 1-32.
- (1954a) Die Übertragung blühfördernder Prinzipien bei Pisum sativum durch Pfropfung. Z. Bot. 42 : 125-34.
- (1954b) Die stoffliche Beeinflussung der Blütenbildung bei Pisum sativum. I Die Wirkung der Stickstoffernährung. Ber. Deut. Bot. Ges. 67 : 75-83.
- (1955) Die stoffliche Beeinflussung der Blütenbildung bei Pisum sativum. II Die Wirkung der Zuckerernährung. Ber. Deut. Bot. Ges. 67 : 107-20.
- (1956) Förderung der Blütenbildung durch Hemmung der vegetativen Entwicklung. Planta 46 : 403-7.
- (1957) Photoperiodische Reaktion bei einer als tagneutral geltenden Sorte von Pisum sativum. Ber. Deut. Bot. Ges. 70 : 191-8.
- (1958) Die Blütenbildung bei Pisum sativum. Weitere Untersuchungen zur Übertragbarkeit eines Blühimpulses durch Pfropfung. Z. Bot. 46 : 242-56.

- Haupt, W. (1969) *Pisum sativum*. In "The Induction of Flowering : Some Case Histories". (Ed. L.T.Evans). Macmillan and Co. : Melbourne. pp.393-408.
- Hoshino, Y. (1915) On the inheritance of the flowering-time in peas and rice. Jour. Coll. Agr. Hokkaido Imp. Univ. Sapporo 6 : 229-288.
- Johnston, M.J. and Crowden, R.K. (1967) Cotyledon excision and flowering in Pisum sativum. Aust. J. biol. Sci. 20 : 461-3.
- Kiyosawa, S. and Kiyosawa, K. (1962) A study of varietal difference in flowering habits of soybean plants as followed by grafting experiments. Plant Cell Physiol. (Tokyo), 3 : 263-273.
- Knave, D.E. (1967) A daylength-sensitive, tall-mutant pea. J. of Heredity 58 : 78-80.
- Köhler, G.D. (1965) Die Physiologie der Blütenbildung bei Pisum sativum. Z. Pflanzenphysiol. 53 : 429-451.
- Lamprecht, H. (1956a) Pisum sativum L. oder P. arvense L. Eine nomenklatorische Studie auf genetischer Basis. Agri Hort. Genet. 14 : 1-4.
- (1956b) Ein Pisum-Typ mit grundständigen Infloreszenzen. Agri Hort. Genet. 14 : 195-202.
- (1961) Die Genenkarte von Pisum. Agri Hort. Genet. 19 : 360-401.
- Lock, R.H. (1907) The present state of knowledge of heredity in Pisum. Ann. Roy. Bot. Gard. Peradenyia. 4 : 93-111.
- Marx, G.A. (1968) Influence of genotype and environment on senescence in peas Pisum sativum L. BioScience 18 : 505-6.
- (1969) Some photo-dependent responses in Pisum. I Physiological behavior. Crop Sci. 9 : 273-6.

- Marushige, K. and Marushige, Y. (1962) An electrophoretic study of tissue extracts from leaf and flower in Pharbitis nil Chois. Plant. Cell Physiol. (Tokyo) 3 : 319-22.
- Mendel, G. (1865) Versuche über Pflanzenhybriden. Verhand. Naturf. Ver. Brunn. 4 : 3-47 (English transl.)
- Moore, T.C. (1964) Effects of cotyledon excision on the flowering of five varieties of Pisum sativum. Pl. Physiol., Lancaster. 39 : 924-7.
- Nitsan, J. (1962) Electrophoretic patterns of Xanthium leaf extracts as affected by physiological age of leaf, photoperiod, and age of plant. Plant Physiol. 37 : 291-5.
- Oppenheim. J.D. (1921) De erfelijkheid van het vroeg of laat bloeien bij Erwten. Ver. t. Bev. van Wetensch. Teelt, Ber. a. d. Leden No. 10 : 2-5 (Citation from Bot. Abst. & Wellensiek (1925b)).
- Paton, D.M. and Barber, H.N. (1955) Physiological genetics of Pisum I. Grafting experiments between early and late varieties. Aust. J. biol. Sci. 8 : 231-240.
- Paton, D.M. (1956) Physiological genetics of Pisum. Ph.D. Thesis, University of Tasmania.
- (1969) Vernalisation, photoperiodic induction, and flower initiation in the late pea cultivar Greenfeast. Aust. J. biol. Sci. 22: 303-10.
- Raghavan, V. and Jacobs, W.P. (1961) Studies on the floral histogenesis and physiology of Perilla. II. Floral induction in cultured apical buds of P. frutescens. Amer. J. Bot. 48 : 751-60.
- Rasmusson, J. (1935) Studies on the inheritance of quantitative characters in Pisum I. Preliminary note on the genetics of time of flowering. Hereditas. 20 : 161-80.

- Resende, F. (1949) Auxin and antiauxin, the hormones responsible for the change of the vegetative into floral phenotypes. Bol. Soc. Portug. Cien. Nat. 2^a S. 2 : 174.
- (1959) On the transmission of the floral state through grafting, from LSDP - or SDP-donors to LSDP - acceptors in LD and SD. Evidence for the separation of growth-hormones from antigrowth-hormones in this transmission. Portug. Acta Biol., (A) 6 : 1-17.
- Rowlands, D.G. (1964) Genetic control of flowering in Pisum sativum L. Genetica 35 : 75-94.
- Schwabe, W.W. (1956) Evidence for a flowering inhibitor produced in long days in Kalanchoe blossfeldiana Ann. Bot. (NS) 20 : 1-14.
- (1959) Studies of long-day inhibition in short-day plants. Jour. Exptl. Bot. 10 : 317-29.
- Skoog, F., and Miller, C.O. (1957) Chemical regulation of growth and organ formation in plant tissue. Symp. Soc. Exp. Biol. 11 : 118 - 131. ed. H.K. Porter.
- Sprent, J.I. and Barber, H.N. (1957) Leaching of a flower inhibitor from late varieties of peas. Nature, Lond. 180 : 200-201.
- Sprent, J.I. (1958) Some aspects of developmental physiology in peas. Ph.D. Thesis, University of Tasmania.
- (1966) The role of the leaf in flowering of late pea varieties. Nature, Lond. 209 : 1043-4.
- Tedin, H. (1897) Några synpunkter vid förädling av ärtor. Sveriges Utsädes föris Tidskrift. 7 : 111.
- Tedin, H. and Tedin, O. (1923) Contributions to the genetics of Pisum III. Internode length, stem thickness and place of the first flower. Hereditas 4 : 351-362.

- Thompson, P.A. and Guttridge, C.G. (1959) Effect of gibberellic acid on initiation of flowers and runners in the strawberry.
Nature, Lond., 184 B.A. 72 - B.A. 73.
- Tschermak, E. von. (1910) Ueber die Vererbung der Blutezeit bei Erbsen.
Verh. Naturf. Ver. Brunn. 49 : 169 - 191.
- Wellensiek, S.J. (1925a) Pisum - crosses. I. Genetica 2 : 1 - 64.
(1925b) Genetic monograph on Pisum. Bibliog. Genet.
2 : 343-476.
- Zeevaart, J.A.D. (1963) Climatic control of reproductive development.
In "Environmental Control of Plant Growth". (Ed. L.T. Evans).
pp 289 - 310. (Academic Press, Inc.: New York.)